Early career achievement award: supplementing omega-6 fatty acids to enhance early embryonic development and pregnancy establishment in *Bos indicus* and *B. taurus* beef cows¹

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ABSTRACT: Our research group investigated the impacts of supplementing Ca salts of soybean oil (CSSO), a source of omega-6 fatty acids (FAs), on reproductive performance of beef cows. Initial studies were conducted with Nelore (*Bos indicus*) cows grazing tropical pastures. Cows were assigned to fixed-time artificial insemination (AI) and supplemented or not with 100 g/d (as-fed basis) of CSSO, and supplementation regimens ranged from days -11 to 28 relative to AI. Overall, CSSO supplementation during the 21 d after AI increased ($P < 0.01$) pregnancy rates from 38.1% (623/1,635 as pregnant/total nonsupplemented cows) to 49.0% (843/1,720 as pregnant/total CSSO-supplemented cows), and these outcomes were associated with enhanced early embryonic development and pregnancy establishment when omega-6 FA were supplemented. To verify this rationale, our group compared FA incorporation in grazing Nelore cows ($n = 90$) supplemented or not with CSSO (100 g/d; as-fed basis) beginning at fixed-time AI until slaughter at day 19 of gestation. Supplementing CSSO increased ($P \leq 0.05$) incorporation of linoleic acid and its omega-6 derivatives in plasma, endometrium, corpus luteum, and conceptus, whereas the same responses were not observed ($P \geq 0.25$) for

omega-3 FA. Complementing these findings, grazing Nelore cows ($n = 100$) were supplemented or not with CSSO (100 g/d; as-fed basis) beginning at fixed-time AI, and assigned to transcervical uterine flush on day 15 of gestation. Supplementing CSSO increased ($P \leq 0.04$) conceptus length (2.58 vs. 1.15 cm) and mRNA expression of *interferon-tau* (4.1-fold increase) and *prostaglandin E synthase 2* (2.6-fold increase), which are critical regulators of pregnancy establishment. These outcomes were recently replicated in *B. taurus* beef cows consuming temperate forages. Pregnancy rates were greater ($P = 0.01$) in Angus cows receiving CSSO (100 g/d; as-fed basis) for 21 d after fixed-time AI (60.2%; 226/383 as pregnant/total cows) compared with nonsupplemented cows (51.7%; 193/388 as pregnant/total cows). Supplementing CSSO to Angus $\times$ Hereford cows ($n = 96$) beginning after AI also increased ($P = 0.05$) mRNA expression of *interferon-tau* in day 15 conceptuses (1.8-fold increase). Collectively, our research demonstrated that post-AI CSSO supplementation favors incorporation of omega-6 FA into maternal and embryonic tissues, which enhances *interferon-tau* synthesis by the conceptus and increases pregnancy rates to fixed-time AI in *B. indicus* and *B. taurus* beef cows.

Key words: beef cows, Ca salts of soybean oil, omega-6 fatty acids, pregnancy

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J. Anim. Sci. 2019.97:485–495

doi: 10.1093/jas/sky414

¹Based on the Early Career Awardee presentation at the 2018 ASAS-CSAS Annual Meeting, July 8 to 12, 2018, Vancouver, BC, Canada.

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Received September 20, 2018.

Accepted October 19, 2018.

INTRODUCTION

According to the United Nations (FAO, 2009), worldwide beef production must increase 120% by 2050 to feed an additional 2.3 billion

people. Resources for beef and other agricultural systems, however, will become even more limited as the planet population increases and urban areas expand. Therefore, efficiency in beef production needs to advance significantly during the next decades to meet the increasing beef demand while fostering ecological stewardship and judicious use of limited natural resources.

Cow-calf production systems are the foundation for beef industries worldwide, regulating the number of cattle available for beef production. In the United States, cow-calf systems represent 86% of beef operations and 84% of the beef cattle population (NASS, 2012). Reproduction is the main factor limiting productivity in cow-calf systems, and pregnancy loss has been recognized as one of the main reproductive challenges in cattle (Zavy, 1994). More specifically, 90% to 100% of fertile beef females effectively conceive after a single service, whether natural service or artificial insemination (AI) is utilized (Sreenan and Diskin, 1983). Yet, <70% of these females remain pregnant 30 d after service, and even fewer females give birth to a live calf (Geary, 2005). The majority of pregnancy losses in cattle occur during the first 27 d of the pregnancy and are classified as early embryonic loss (Humbolt, 2001). Hence, management strategies to promote early embryonic survival are warranted for optimal reproductive and overall efficiency of cow-calf operations, and to ensure adequate supply of cattle to meet the global demand for beef. These include nutritional interventions that benefit embryo development, such as fatty acid (FA) supplementation to beef cows (Hess et al., 2008).

SUPPLEMENTAL FAT

Fat supplementation has been extensively evaluated during the last decades as a manner to enhance reproductive efficiency in beef cattle. These research studies considered supplemental fat as an energy source, nutraceutical ingredient, or both, and focused on pre-pubertal, pubertal, prepartum, and postpartum females (Funston, 2004; Hess et al., 2008). Moreover, most research groups focused on the nutraceutical roles of linoleic and linolenic acids, based on the initial reports suggesting that these essential FA directly modulate cattle reproductive function (Staples, 1998; Williams and Stanko, 2000; Hess et al., 2005).

Several studies investigating postpartum fat supplementation indicated that supplementing linoleic acid was detrimental, whereas linolenic acid was beneficial to reproductive performance of lactating

beef females (Hess et al., 2008). These outcomes were mainly attributed to the role of these FA on prostaglandin (PG) $F_{2\alpha}$ synthesis, which triggers luteolysis and has direct embryotoxic effects during early gestation (Inskip, 2004). Linolenic acid is a precursor of eicosapentaenoic and docosahexaenoic acids, which inhibit cyclooxygenase activity and consequently $PGF_{2\alpha}$ synthesis (Yaqoob and Calder, 2007). Accordingly, supplementation with linolenic acid was associated with reduced pregnancy losses in lactating dairy cattle (Burke et al., 1997; Thatcher et al., 1997; Mattos et al., 2000). In turn, linoleic acid can be desaturated and elongated to arachidonic acid, and serves as a precursor for $PGF_{2\alpha}$ (Yaqoob and Calder, 2007). Hence, linoleic acid supplementation has been associated with increased $PGF_{2\alpha}$ synthesis, impaired luteal function, reduced pregnancy rates (Grant et al., 2003; Grant et al., 2005), and not recommended as a nutritional strategy to improve pregnancy rates of postpartum beef females (Hess et al., 2008). These aforementioned studies, however, evaluated linoleic acid supplementation throughout the postpartum period, which includes not only early pregnancy development but also other reproductive activities known to be modulated by dietary fat and PG, such as estrus resumption, fertility, and ovulation parameters (Mattos et al., 2000; Funston, 2004; Hess et al., 2008).

FEEDING ESSENTIAL FAS DURING EARLY GESTATION

Differing from the results compiled by Hess et al. (2008), our research group reported that supplementing soybean oil (rich in linoleic acid; Table 1) to beef cows beginning after AI increased pregnancy rates by 30% (Lopes et al., 2009, 2011). These initial projects were completed with Nelore (*Bos indicus*) cows grazing tropical forages, and supplemented with soybean oil as Ca salts (CSSO) to minimize ruminal bio-hydrogenation (Sukhija and Palmquist, 1990). Lopes et al. (2009) conducted four different trials supplementing 100 g/d of CSSO (as-fed basis) to beef cows around the time of breeding. These authors reported increased pregnancy rates to fixed-time AI (day 0 in all experiments) in lactating primiparous cows supplemented with CSSO from the beginning of estrus synchronization protocol (day -11) until day 28 (Table 2; Exp. A1). Similar outcomes were observed in lactating primiparous cows supplemented with CSSO from day -11 until day 28 compared with cohorts supplemented from days -11 to 16 or nonsupplemented cows (Table 2; Exp.

Table 1. Nutritional and fatty acid profile (DM basis) of commercial supplements based on Ca salts of soybean oil

Item	Megalac-E ¹	Essentiom ²
Total digestible nutrients, %	193	190
Net energy for maintenance, Mcal/kg	6.97	4.86
Crude protein, %	0.80	0.72
Neutral detergent fiber, %	1.15	0.91
Fatty acids ² , %	87.8	82.5
Palmitic (16:0), %	15.5	26.5
Stearic (18:0), %	4.50	3.33
Oleic (18:1), %	27.5	22.6
Linoleic (18:2), %	35.8	25.4
Linolenic (18:3), %	2.89	2.57

¹Elanco Saúde Animal (São Paulo, Brazil). Used by [Lopes et al. \(2009, 2011\)](#), [Cooke et al. \(2014a\)](#), and [Cipriano et al. \(2016\)](#). Values reported were averaged from [Cooke et al. \(2014a\)](#) and [Cipriano et al. \(2016\)](#).

²Church and Dwight Co., Inc. (Princeton, NJ). Values based on [Brandão et al. \(2018\)](#).

A2). [Lopes et al. \(2009\)](#) also reported a trend for increased pregnancy rates in nulliparous and multiparous lactating cows supplemented with CSSO from the end of synchronization protocol (day 0) until 21 d after fixed-time embryo transfer (day 28; Exp. A3). Finally, [Lopes et al. \(2009\)](#) compared pregnancy rates to fixed-time AI in lactating multiparous cows supplemented (as-fed basis) with 100 g/d of CSSO or with 100 g/d of Ca salts of palm oil (iso-caloric and iso-lipidic supplement based on saturated fat) from days 0 to 28, and again reported greater pregnancy rates in CSSO-supplemented cows ([Table 2](#); Exp. A4). Together, these results were the initial evidence that CSSO supplementation benefits cattle reproduction, particularly during early gestation, and such outcomes were independent of its contribution to energy intake.

[Lopes et al. \(2011\)](#) further investigated the impacts CSSO supplementation to Nelore beef

Table 2. Pregnancy rates in *Bos indicus* beef cows supplemented or not with Ca salts of soybean oil (CSSO)^{1,2}

Item	Pregnancy rate, %	P-values
Lopes et al. (2009)		
<i>Exp. A1 (artificial insemination on day 0)</i>		
Control	39.6 (182/459)	0.04
CSSO from days −11 to 28	51.2 (231/451)	
<i>Exp. A2 (artificial insemination on day 0)</i>		
Control	31.3 (82/262) ^b	0.05
CSSO from days −11 to 16	35.8 (108/302) ^b	
CSSO from days −11 to 28	42.9 (109/254) ^a	
<i>Exp. A3 (embryo transfer on day 7)</i>		
Control	37.7 (78/207)	0.07
CSSO from days 0 to 28	49.6 (113/228)	
<i>Exp. A4 (artificial insemination on day 0)</i>		
SFA from days 0 to 28	35.5 (85/239)	0.02
CSSO from days 0 to 28	47.9 (127/265)	
Lopes et al. (2011)		
<i>Exp. B1 (artificial insemination on day 0)</i>		
Control	41.6 (65/156)	0.22
SFA from days 0 to 14	41.9 (67/160)	
SFA from days 0 to 21	42.7 (67/157)	
SFA from days 0 to 28	41.3 (64/155)	
CSSO from days 0 to 14	43.1 (131/304)	
CSSO from days 0 to 21	51.0 (128/251)	
CSSO from days 0 to 28	49.8 (135/271)	
<i>Exp. B2 (artificial insemination on day 0)</i>		
CSSO from days 0 to 14	33.1 (42/127) ^{ab}	0.05
CSSO from days 0 to 21	50.3 (78/155) ^a	
CSSO from days 7 to 21	45.3 (58/128) ^{ab}	
CSSO from days 14 to 21	42.8 (39/91) ^{ab}	

¹Adapted from [Lopes et al. \(2009, 2011\)](#). Values with different superscripts differ ($P < 0.05$). Values in parentheses represent number of pregnant cows per total cows.

²Across experiments, cows offered daily a protein-mineral mix with the addition (as-fed basis) of 100 g/d of CSSO, 100 g/d of kaolin (rumen-inert indigestible substance; control), or 100 g/d of Ca salts of palm oil (SFA).

cows, with intent of determining the critical post-breeding period for CSSO feeding. In Exp. B1, lactating multiparous cows were assigned to receive several treatment combinations of FA source (non-supplemented, 100 g/d of CSSO, or 100 g/d Ca salts of palm oil; as-fed basis) for 14, 21, or 28 d after fixed-time AI. Pregnancy rates were the greatest in cows receiving CSSO for at least 21 d compared with all other treatments combined (Table 2; 50.4% vs. 42.4%, respectively). In Exp. B2, lactating multiparous cows were assigned to receive 100 g/d of CSSO (as-fed basis) during different durations of time after fixed-time AI (day 0), including days 0 to 14, 0 to 21, 7 to 21, and 14 to 21. Lopes et al. (2011) reported that pregnancy rates were the greatest in cows receiving CSSO for 21 d beginning at fixed-time AI (Table 2), and recommended this supplementation schedule to maximize the reproductive benefits of CSSO. Nevertheless, Lopes et al. (2011) recognized that the biological mechanisms by which CSSO modulated reproductive function during early gestation were still unknown, but were likely associated with embryonic development and early pregnancy establishment.

INCORPORATION OF FATTY ACIDS BY THE REPRODUCTIVE TRACT

The CSSO supplemented by Lopes et al. (2009, 2011) contained mostly linoleic acid (Table 1) and trace amount of linolenic acid. These research efforts, however, did not evaluate FA incorporation into blood and reproductive tissues to attribute the reproductive benefits of CSSO with supplemental linoleic, linolenic, or both FA. Moreover, Lopes et al. (2009, 2011) did not evaluate potential mechanisms by which CSSO supplementation impacted early pregnancy development. Previous research established that supplemental FA alters FA profile in reproductive tissues of cows, including the corpus luteum and endometrium (Burns et al., 2003; White et al., 2012), and modulates hormones associated with early pregnancy establishment such as PG (Hawkins et al., 1995; Grant et al., 2005). Therefore, the next step was to evaluate FA incorporation into reproductive tissues and investigate biological mechanisms associated with pregnancy establishment in *B. indicus* cows supplemented with CSSO after fixed-time AI.

In Cooke et al. (2014a), nonlactating multiparous Nelore cows were supplemented (as-fed basis) with 100 g/d of CSSO or kaolin (rumen-inert indigestible substance) beginning after fixed-time AI, and slaughtered 19 d after AI for collection

of reproductive tissues. Supplementing CSSO increased incorporation of linoleic acid and its omega-6 derivatives into plasma, endometrium, corpus luteum, and conceptus, whereas the same responses were not observed for linolenic acid and its omega-3 derivatives (Table 3). These results indicated that CSSO supplementation 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 omega-6 FA to the conceptus. As previously discussed, omega-6 FA are precursors for PGF_{2α} synthesis (Yaqoob and Calder, 2007), the hormone responsible for luteolysis and pregnancy termination (Senger, 2003). Moreover, linoleic acid also can be converted into eicosadienoic acid rather than arachidonic acid (Cooke et al., 2014a), which is known to reduce PGF_{2α} synthesis (Staples et al., 1998; Cheng et al., 2001). Research also demonstrated that PG from the two series, such as PGE₂ and PGI₂, originated from omega-6 FA (Schmitz and Ecker, 2008), is critical regulators of conceptus elongation and pregnancy establishment in ruminants (Erdem and Guzeloglu, 2010; Dorniak et al., 2011). These PG is synthesized by the conceptus and endometrium, modulate synthesis and endometrial activity of *interferon-tau* (IFNT), and seems to be fundamental for conceptus development and pregnancy signaling to maternal tissues (Erdem and Guzeloglu, 2010; Dorniak et al. 2011). Thus, it became plausible to conclude that pregnancy rates were greater in CSSO-supplemented cows in Lopes et al. (2009, 2011) resulting from incorporation of omega-6 FA, and not omega-3 FA, into their reproductive tissues and conceptus during early gestation (Cooke et al., 2014a).

IMPACTS ON EARLY PREGNANCY ESTABLISHMENT

Cooke et al. (2014a) also investigated the effects of CSSO supplementation on factors associated with embryonic development or early pregnancy establishment. These included conceptus size, mRNA expression of genes associated with oxytocin, progesterone, and PG in endometrial and luteal samples, as well as mRNA expression of *IFNT* in the conceptus. In fact, the hypothesis stated by Cooke et al. (2014a) revolved around the IFNT-signaling cascade (Thatcher et al., 1995) as the key mechanism responsible for increased pregnancy rates in CSSO-supplemented cows (Lopes et al., 2009; Lopes et al., 2011). Supplemental

Table 3. Concentrations of fatty acids (FAs) in samples collected from *Bos indicus* beef cows receiving (as-fed basis) 100 g/d of Ca salts of soybean oil (CSSO) or 100 g/d of kaolin (control; rumen-inert indigestible substance) beginning after fixed-time artificial insemination, and slaughtered 19 after insemination for tissue collection¹

Item	Plasma ²	Endometrium ³	Corpus luteum ³	Conceptus ³
<i>Linoleic (18:2 n-6)</i>				
CSSO	0.540	0.358	7.035	0.174
Control	0.275	0.144	3.935	0.022
SEM	0.021	0.043	0.543	0.060
<i>P</i>	<0.01	<0.01	<0.01	0.08
<i>Arachidonic (20:4 n-6)</i>				
CSSO	0.025	0.266	4.942	0.312
Control	0.023	0.241	4.731	0.086
SEM	0.004	0.061	0.349	0.044
<i>P</i>	0.74	0.77	0.67	<0.01
<i>Total omega-6</i>				
CSSO	0.565	0.938	17.78	2.045
Control	0.296	0.549	12.72	0.384
SEM	0.022	0.136	1.197	0.755
<i>P</i>	<0.01	0.05	<0.01	0.13
<i>Linolenic (18:3 n-3)</i>				
CSSO	0.136	0.016	0.615	0.029
Control	0.143	0.014	0.729	0.035
SEM	0.005	0.002	0.070	0.016
<i>P</i>	0.41	0.53	0.25	0.80
<i>Total omega-3</i>				
CSSO	0.136	0.637	5.359	1.923
Control	0.143	0.433	6.044	1.439
SEM	0.005	0.090	0.488	0.578
<i>P</i>	0.41	0.12	0.32	0.56
<i>Total polyunsaturated FA</i>				
CSSO	0.701	1.576	23.16	3.967
Control	0.439	0.983	18.76	1.824
SEM	0.025	0.221	1.625	0.950
<i>P</i>	<0.01	0.05	0.05	0.13
<i>Total FA</i>				
CSSO	1.966	6.181	59.60	14.13
Control	1.693	4.221	54.39	8.883
SEM	0.045	0.863	3.320	2.450
<i>P</i>	<0.01	0.12	0.27	0.15

¹Adapted from [Cooke et al. \(2014a\)](#).²Samples collected on days 0, 7, and 18 relative to AI (day 0). Values obtained on day 0 served as covariate; therefore, values are covariately adjusted means and reported as milligram per milliliter of plasma.³Samples collected on day 19 relative to AI (day 0). Values are reported as milligram per gram of tissue.

CSSO, however, did not impact any of these aforementioned variables (Table 4), despite a tendency for increased IFNT concentration in uterine flushes collected from CSSO-supplemented cows. As noted by [Cooke et al. \(2014a\)](#), cows were slaughtered 19 d 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 allow recovery of conceptuses that still expressed *IFNT* mRNA ([Roberts et al.,](#)

[1992](#)) and provided enough tissue for both FA and mRNA expression analyses. Yet, the physiological processes responsible for pregnancy signaling to maternal tissues occur approximately on days 15 to 17 of gestation ([Mann et al., 1999](#); [Ealy et al., 2001](#)). Hence, reproductive tissues and conceptuses were collected after the critical period for pregnancy recognition, which likely prevented proper assessment of treatment effects on expression of luteal, endometrial, and conceptus genes associated with pregnancy development. [Cooke et al. \(2014a\)](#) stated

Table 4. Conceptus development and mRNA expression of genes associated with pregnancy establishment and maintenance in *Bos indicus* beef cows receiving (as-fed basis) 100 g/d of Ca salts of soybean oil (CSSO) or 100 g/d of kaolin (control; rumen-inert indigestible substance) beginning after fixed-time artificial insemination, and slaughtered 19 after insemination for tissue collection¹

Item	CSSO	Control	SEM	P
<i>Conceptus dimension</i>				
Length, cm	33.4	34.7	3.3	0.78
Weight, mg	0.239	0.243	0.036	0.93
Interferon-tau in uterine flushing media, ng/mL	10.95	7.29	1.47	0.09
<i>mRNA expression²</i>				
Corpus luteum				
<i>3β-Hydroxysteroid dehydrogenase</i>	2.45	2.65	0.30	0.63
<i>Steroidogenic acute regulatory protein</i>	1.68	1.90	0.12	0.22
<i>Oxytocin</i>	3.11	2.28	0.35	0.12
<i>Prostaglandin receptor</i>	2.44	2.11	0.38	0.56
Endometrium				
<i>Cyclooxygenase-2</i>	23.97	40.98	9.95	0.24
<i>Oxytocin receptor</i>	23.98	31.56	7.01	0.45
Conceptus				
<i>Interferon-tau</i>	2.74	2.93	0.33	0.68

¹Adapted from [Cooke et al. \(2014a\)](#).

²Values are expressed as relative fold change compared with threshold cycle of reference genes analyzed within the same sample ([Ocón-Grove et al., 2008](#)).

that research was still warranted to understand the reproductive benefits of CSSO supplementation during early gestation, particularly mRNA expression of genes within the PG two series and IFN-cascade during the critical period for pregnancy recognition (i.e., day 15 of gestation).

To complement [Cooke et al. \(2014a\)](#) and provide further biological support to [Lopes et al. \(2009, 2011\)](#), [Cipriano et al. \(2016\)](#) supplemented lactating Nelore cows with 100 g/d of CSSO or kaolin (as-fed basis) beginning after fixed-time AI. On day 15 of after AI, a subset of cows were assigned to conceptus collection via transcervical flushing with saline followed by endometrial biopsy in the uterine horn ipsilateral to the corpus luteum. In cows not assigned to conceptus collection, blood samples were collected for whole blood RNA extraction on day 20 after AI, and pregnancy status was verified by transrectal ultrasonography on day 30 after AI. Supplementing CSSO increased length and mRNA expression of *prostaglandin E synthase* and *IFNT* by the conceptus, as well as blood mRNA expression of interferon-stimulated genes (ISG) on day 20 ([Table 5](#)). Accordingly, mRNA expression of ISGs has been used to gauge IFNT production and conceptus development from days 15 to 22 of gestation ([Fricke et al. 2016](#)), given that IFNT synthesis upregulates mRNA expression of ISGs in circulating blood leukocytes ([Stevenson et al., 2007; Gifford et al., 2008; Green et al., 2010](#)). In turn, CSSO supplementation did not impact endometrial

mRNA expression of *prostaglandin E synthase* and *cyclooxygenase-2* ([Table 5](#)), suggesting that CSSO effects on PG-related responses on day 15 of gestation may be specific to the conceptus. Collectively, [Cipriano et al. \(2016\)](#) demonstrated that CSSO supplementation enhanced conceptus development and IFNT synthesis during the pregnancy recognition period ([Spencer and Bazer, 2004; Fricke et al. 2016](#)), which explains the increased pregnancy rates to fixed-time AI when CSSO is supplemented to *B. indicus* beef cows during early gestation ([Lopes et al., 2009, 2011](#)).

WHAT ABOUT *BOS TAURUS* CATTLE?

Hitherto, research from our group demonstrated that supplementing CSSO to beef cows after AI enhanced pregnancy establishment by increasing conceptus development and signaling via the IFNT cascade ([Cipriano et al., 2016](#)), whereas these effects were modulated by omega-6 FA ([Cooke et al., 2014a](#)) and resulted in increased pregnancy rates to AI ([Lopes et al., 2009, 2011](#)). These experiments, however, 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, [Brandão et al. \(2018\)](#) conducted two experiments

Table 5. Conceptus development and mRNA expression of genes associated with pregnancy establishment and maintenance in *Bos indicus* beef cows receiving (as-fed basis) 100 g/d of Ca salts of soybean oil (CSSO) or 100 g/d of kaolin (control; rumen-inert indigestible substance) beginning after fixed-time artificial insemination (AI)

Item	CSSO	CON	SEM	P-values
Conceptus length, cm	2.58	1.15	0.59	0.04
Conceptus, mRNA expression ²				
Interferon- τ	21.0	5.1	5.0	0.03
Prostaglandin E synthase	7.89	2.99	1.49	0.02
Endometrium, mRNA expression ²				
Cyclooxygenase-2	6.90	4.89	1.50	0.37
Prostaglandin E synthase	5.34	4.21	0.76	0.30
Blood cells, mRNA expression ^{2,3}				
Interferon-stimulated gene 15	33.8	23.7	2.6	<0.01
Myxovirus resistance 2	47.1	27.6	4.2	<0.01
20,50-oligoadenylate synthetase	48.1	35.2	3.8	0.02

Conceptus collection and endometrial biopsy were performed on day 15 after AI.

¹Adapted from Cipriano et al. (2016).

²mRNA expression values are expressed as relative fold change compared to threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).

³Blood samples were collected on day 20 after AI from cows not assigned to conceptus collection. Only results from cows diagnosed as pregnant on day 30 after AI, via transrectal ultrasonography (5.0 MHz transducer; 500 V, Aloka, Wallingford, CT), are reported.

to replicate these outcomes in *B. taurus* cattle in typical U.S. conditions.

In Exp. C1, Brandao et al. (2018) supplemented lactating multiparous Angus cows with 100 g/d of CSSO or 87 g/d of a prilled saturated fat source (as-fed basis), which served as iso-caloric and iso-lipidic control to CSSO, for 21 d after fixed-time AI. Note that the CSSO used by Brandao et al. (2018) differed slightly from the CSSO previously used, although both had linoleic acid as the major FA component (Table 1). Similar to the findings in *B. indicus* cows, supplementing CSSO increased pregnancy rates to fixed-time AI by ~17% (Table 6) in *B. taurus* females. According to 2017 average prices of the CSSO source (\$2.65/kg) and feed ingredients in the United States (\$0.39 per kilogram of soybean meal and \$0.18 per kilogram of corn; USDA-Agricultural Marketing Service, 2017), the cost of purchasing the CSSO-based supplement used by Brandão et al. (2018) to feed 300 cows for 21 d was \$1,997. It should be noted that corn, soybean meal, and other grain mixtures were used as carrier, at a low daily intake rate (≤ 0.4 kg/cow daily; as-fed basis), for the CSSO, kaolin, and saturated fat sources across all experiments reported herein. The increase in pregnancy rates to timed-AI observed with CSSO supplementation would result in 26 more pregnancies by 300 cows. Assuming that all cows would calve and wean a 230-kg calf at 7 mo of age, supplementing CSSO would increase weaning returns by \$21,528 (US\$ 3.6 per kilogram of BW across genders, USDA-Agricultural

Marketing Service, 2017), resulting in a return on investment of 1,078% based on feed purchase only. Additional expenses associated with labor and feeding logistics were not included, as these vary according to operation, cowherd size, location, and many other factors. In turn, additional revenues associated with increased AI-sired calves in CSSO-supplemented herds, including greater calf genetic potential, age, and BW at weaning (Cooke et al., 2014b), were also not included in the previous calculation. Predicting such outcomes would be highly speculative, whereas research is warranted to document the benefits of CSSO supplementation during the breeding season in herds that used fixed-time AI and/or natural breeding. Yet, results from Brandão et al. (2018) clearly indicate that post-AI CSSO supplementation may be an alternative to economically benefit commercial cow-calf systems.

In Exp. C2, Brandão et al. (2018) supplemented lactating multiparous Angus $\times$ Hereford cows as in Exp. C1, and adopted a similar research design as in Cipriano et al. (2016). Cows supplemented with CSSO had greater plasma concentrations of linoleic and total omega-6 FA, and reduced plasma concentrations of linolenic and total omega-3 FA (Table 6), corroborating with Cooke et al. (2014a) that CSSO supplies linoleic acid and its omega-6 derivatives when supplemented to *B. taurus* beef cows. Moreover, CSSO supplementation increased ($P=0.05$) mRNA expression of *IFNT* by the conceptus, and blood mRNA expression of the ISGs *interferon-stimulated gene 15* and *20,50-oligoadenylate*

Table 6. Reproductive responses and plasma fatty acid (FA) concentrations in *Bos taurus* beef cows receiving (as-fed basis) 100 g/d of Ca salts of soybean oil (CSSO) or 87 g/d of prilled saturated fat source (iso-caloric and iso-lipidic control to CSSO) beginning after fixed-time artificial insemination (AI)¹

Item	CSSO	CON	SEM	P-values
<i>Exp. C1</i>				
Pregnancy rate ² , %	60.2 (226/383)	51.7 (193/388)	4.2	0.01
<i>Exp. C2</i>				
Plasma FA ³ , mg/mL				
Linoleic (18:2 n-6)	0.223	0.149	0.005	<0.01
Total omega-6	0.263	0.192	0.008	<0.01
Linolenic (18:3 n-3)	0.094	0.114	0.003	<0.01
Total omega-3	0.105	0.123	0.003	<0.01
Total polyunsaturated FA	0.371	0.311	0.011	<0.01
Total FA	0.679	0.642	0.021	0.24
Conceptus length, cm	11.3	11.4	3.1	0.97
Conceptus, mRNA expression ⁴				
<i>Interferon-tau</i>	21.3	12.1	3.4	0.05
<i>Prostaglandin E synthase</i>	2.22	2.50	0.48	0.69
Endometrium, mRNA expression ⁴				
<i>Cyclooxygenase-2</i>	4.88	5.11	1.32	0.89
<i>Prostaglandin E synthase</i>	5.76	7.40	1.10	0.30
Blood cells, mRNA expression ^{4,5}				
<i>Interferon-stimulated gene 15</i>	43.1	29.8	4.6	0.04
<i>Myxovirus resistance 2</i>	20.2	20.1	2.7	0.98
<i>20,50-Oligoadenylate synthetase</i>	26.8	18.3	2.7	0.03

¹In Exp. I, pregnancy status was verified 45 to 55 d after AI transrectal ultrasonography (5.0 MHz linear transducer, Ibex Pro, E.I. Medical Imaging, Loveland, CO). In Exp. II, conceptus collection and endometrial biopsy were performed on day 15 after AI. Adapted from [Brandão et al. \(2018\)](#).

²Values in parentheses represent number of pregnant cows per total cows.

³Samples collected on days 0, 7, and 15 relative to AI (day 0). Values obtained on day 0 served as covariate; therefore, values are covariately adjusted means and reported as milligram per milliliter of plasma.

⁴mRNA expression values are expressed as relative fold change compared to threshold cycle of reference genes analyzed within the same sample ([Ocón-Grove et al., 2008](#)).

⁵Blood samples were collected on day 20 after AI from cows not assigned to conceptus collection. Only results from cows diagnosed as pregnant on day 30 after AI, via transrectal ultrasonography (5.0 MHz transducer; 500 V, Aloka, Wallingford, CT), are reported.

synthetase on day 20 in gestating cows ([Table 6](#)). Differing than [Cipriano et al. \(2016\)](#), CSSO supplementation did not increase conceptus length and mRNA expression of *prostaglandin E synthase* in the conceptus ([Table 6](#)). Alternatively, conceptus length across treatments was 11.4 ± 1.9 cm in [Brandão et al. \(2018\)](#) and 2.4 ± 0.5 cm in [Cipriano et al. \(2016\)](#). These results indicate that *B. taurus* conceptus are at an advanced stage of elongation compared with *B. indicus* conceptus on day 15 of gestation, and perhaps past the stage in which CSSO supplementation enhances conceptus growth and modulates mRNA expression of *prostaglandin E synthase*. Nevertheless, results from [Brandão et al. \(2018\)](#) confirmed that post-AI CSSO supplementation to *B. taurus* cows improved pregnancy rates to AI, which was associated with increased mRNA expression of *IFNT* by the conceptus when CSSO is supplemented during early gestation.

SUMMARY AND CONCLUSIONS

A diagram summarizing the impacts of supplementing CSSO to beef cows during early gestation is provided in [Figure 1](#). Collectively, supplementing CSSO increased incorporation of omega-6 FA into maternal and embryonic tissues, promoted IFNT synthesis by the conceptus during the maternal pregnancy recognition period, leading to increased pregnancy success in beef cows. These outcomes were generated across several research trials using nearly 6,000 beef cows from different subspecies and managed in different environments, and were independent of the energy contribution of CSSO given that iso-caloric and iso-lipidic control supplements were included. Differences in CSSO supplementation effects on day 15 conceptuses were noted between subspecies, likely due to hastened embryonic development in *B. taurus* vs. *B. indicus* cattle. Nevertheless, CSSO supplementation is a

Figure 1. Summary diagram about the impacts of supplementing Ca salts of soybean oil (CSSO) beginning after fixed-time artificial insemination (AI) to beef cows (IFNT = *interferon-tau*; PGES = *prostaglandin E synthase*; ISGs = *interferon-stimulated genes*). Adapted from Lopes et al. (2009, 2011), Cooke et al. (2014a), Cipriano et al., (2016), and Brandão et al. (2018).

nutritional alternative to enhance pregnancy establishment and overall reproductive performance of *B. taurus* and *B. indicus* beef cows reared in temperate and tropical environments.

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