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

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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 day 27 of gestation (Cipriano et al., 2016). 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; Lopes et al., 2011). This outcome was credited to enhanced early pregnancy maintenance 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 mRNA expression of interferon-tau (**IFNt**) on day 15 of gestation, which is the signaling molecule responsible for pregnancy recognition by maternal tissues to prevent luteolysis and subsequent pregnancy loss (Spencer and Bazer, 2004).

These experiments, however, were conducted with *B. indicus* cows reared in tropical environments. Pregnancy establishment differs among *B. indicus* and *B. taurus* females (Mercadante et al., 2013), and fatty acid (**FA**) composition also differs among tropical and temperate feed ingredients. Hence, research is needed to validate the aforementioned outcomes in *B. taurus* cows within typical U.S. operations. We hypothesized that CSSO supplementation after timed-AI will increase ω-6 FA 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 utilized 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; Exp. 1), and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee (Exp. 2; #4938).

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Exp. 1

Animals, treatments, and sampling. This experiment (days −10 to 55) was conducted in cow–calf operations (n = 7) managed by the Virginia Department of Corrections, with a total of 771 lactating, multiparous, nonpregnant Angus cows (age = 5.98 ± 0.11 yr, days postpartum = 65.2 ± 0.6 d, and body condition score [BCS] = 5.21 ± 0.03). Across locations, cows were ranked by BCS and days postpartum on day −10 and allocated to a total 22 groups averaging 35 cows each (range = 22 to 50 cows/group). 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 days −10 to −1, groups were supplemented with (as-fed basis) 100 g of ground corn + 100 g of soybean meal per cow daily and enrolled in an estrus-synchronization + fixed-time AI (day 0) protocol (Larson et al., 2006). Multiple AI technicians (n = 11) and semen from different Angus sires (n = 13) were used across locations and groups but balanced between treatments within each location.

Immediately after AI (day 0), groups within each location were assigned randomly to receive the aforementioned supplement mixed with (as-fed basis) the following: 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 (Energy Booster; 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 FA composition (Table 1) and offered from days 0 to 21. Limestone was added to CON to balance treatment Ca content (Table 1). Pregnancy rate to timed-AI was determined between days 45 and 55 after AI via transrectal ultrasonography by the presence of a viable fetus (5.0 MHz linear transducer, Ibex Pro, E.I. Medical Imaging, Loveland, CO).

Exp. 2

Animals and treatments. This experiment (days −10 to 30) was conducted at the Oregon State University—Eastern Oregon Agricultural Research Center (Union station), with 90 lactating, multiparous, nonpregnant Angus × Hereford cows (age = 6.81 ± 0.26 yr, days postpartum = 63.6 ± 1.2 d, body weight = 572.5 ± 5.8 d, and BCS = 5.11 ± 0.04). Cows were ranked by BCS and days postpartum and allocated to 18 pens (five cows/pen) on day −10. Cows received 20 kg/cow daily (DM basis) of grass-alfalfa and ad libitum access to water and mineral mix during the experimental period. From days −10 to 0, cows were enrolled in the same estrus-synchronization + fixed-time AI protocol (Larson et al., 2006) and received supplementation as described in Exp. 1. Cows were inseminated on day 0 by the same technician, using semen from the same Angus bull and batch. Immediately after AI, pens were assigned randomly to receive the same treatments described in Exp. 1 (n = 9 pens/treatment) from days 0 to 21.

Table 1. Nutritional profile (DM basis) of treatments[a]

Item	CSSO	CON
DM, %	92.5	93.1
Total digestible nutrients,[b] %	122	122
Net energy for maintenance,[c] Mcal/kg	3.16	3.23
Crude protein, %	19.9	19.6
Neutral detergent fiber, %	7.60	7.78
Ca, %	3.27	2.81
FAs, %	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

[a]CSSO = daily supplementation (per cow; as-fed basis) with 100 g of a soybean meal + 100 g of ground corn + 100 g of CSSO (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per cow; as-fed basis) 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.

Sampling. 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. 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 dominant follicle diameter (day 0) and estimate corpus luteum (**CL**) volume (days 7 and 15). 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 (two or three cows per pen; CSSO, n = 20; CON, n = 24), were assigned to conceptus collection via transcervical flushing followed by endometrial biopsy in the uterine horn ipsilateral to the CL (Cipriano et al., 2016). All cows returned to their original pens after conceptus collection and endometrial biopsy. On day 20, blood samples were collected from the nonflushed cows (two or three 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 was terminated, and blood samples were collected from the nonflushed cows on day 30 for pregnancy evaluation.

Laboratorial analysis. Blood samples collected on days 0, 7, and 15 were placed immediately on ice, centrifuged (2,500 × *g* for 30 min; 4 °C) for plasma harvest and stored at −20 °C on the same day of collection. Samples were analyzed for FA concentrations (Cipriano et al., 2016). 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 progesterone concentrations using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Plasma samples collected on day 30 were analyzed for pregnancy-associated glycoproteins (BioPRYN; Gold Standard Labs, Bowling Green, KY) for evaluation of pregnancy status.

Conceptus and endometrial samples, as well as blood samples collected in PAXgene tubes were stored as in Cipriano et al. (2016). Total RNA was extracted from tissue samples collected from cows that had a conceptus and from whole blood samples collected on day 20 from cows diagnosed as pregnant on day 30, for mRNA expression analysis by reverse transcription-polymerase chain reaction as described by Cipriano et al. (2016).

Statistical Analyses

Quantitative and binary data were analyzed, respectively, with the MIXED and GLIMMIX procedures of SAS (SAS Institute Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df 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 sire and AI technician as random variables. Data from Exp. 2 used pen as experimental unit, as well as pen (treatment) and cow (pen) as random variables. Model statements contained the effects of treatment, in addition to day and the resultant interaction for repeated measures. Plasma FA concentrations were analyzed using values from day 0 as an independent covariate. The specified term for all repeated statements was day, with cow (pen) as subject, and the covariance structure used was first-order autoregressive based on the smallest Akaike information criterion. Results are reported as least square means 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 AND DISCUSSION

Exp. 1

Cows supplemented with CSSO had greater ($P < 0.01$) pregnancy rates to timed-AI compared with CON cows (60.2% vs. 51.7%, respectively; SEM = 4.2), supporting our hypothesis and corroborating with our previous research in *B. indicus* cattle (Lopes et al., 2009; Lopes et al., 2011).

According to 2017 average prices of the CSSO source (Essentiom; $2.65/kg) and feed ingredients in the Pacific Northwest ($0.39/kg of soybean meal and $0.18/kg of corn; USDA-Agricultural Marketing Service, 2017), the cost of purchasing the CSSO-based supplement used herein to feed 300 cows for 21 days was $1,997. In turn, 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 months of age, supplementing CSSO would increase weaning returns by $21,528 (US$ 3.6/kg of body weight 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 herein, as these vary according to operation, cowherd size, location, and many other factors. Nevertheless, these results indicate that post-AI CSSO supplementation may be an alternative to economically benefit commercial U.S. cow–calf operations.

Exp. 2

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 that cows in both treatments had similar plasma FA concentrations and profile before timed-AI. 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 2). In turn, CON cows had greater ($P \leq 0.02$) mean concentrations of

Table 2. Plasma FA concentrations (μg/mL of plasma) in beef cows supplemented with CSSO (n = 9) or prilled saturated fat (CON; n = 9) in Exp. 2[a]

Item	CSSO	CON	SEM	P value
Palmitoleic (16:1)	5.60	6.62	0.22	<0.01
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
Docosadienoic (22:2, ω-6)	14.5	17.1	0.7	0.03
Total saturated FAs	249	255	10	0.69
Total monounsaturated FAs	64.2	69.8	3.9	0.33
Total polyunsaturated FAs	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 FAs	679	642	21	0.24

[a]Treatments were offered from day 0 (timed-AI) to 21 of the experiment. Blood samples were collected from all cows on days 0, 7, and 15. Values obtained on day 0 served as covariate; therefore, values reported are covariately adjusted means across days 7 and 15.

plasma palmitoleic, oleic, linolenic, docosadienoic, and ω-3 FA compared with CON cows (Table 2). These results corroborate the FA content and profile of the CSSO treatment (predominantly linoleic acid), given that plasma FA concentrations directly reflects intake and duodenal flow of FA (Cooke et al., 2014).

Diameter of the dominant follicle on day 0 was similar (P = 0.14) between CSSO-supplemented and CON cows (Table 3). No treatment differences were detected ($P \geq 0.73$) for plasma progesterone concentration and CL volume during the experiment (Table 3). These results suggest that CSSO supplementation improves reproductive performance (as in Exp. 1) in *B. taurus* cows independently of CL development and circulating progesterone concentrations during early gestation (Cipriano et al., 2016).

A treatment effect was detected for mRNA expression of IFNt, which was greater ($P \leq 0.03$) in conceptuses from CSSO-supplemented vs. CON cows (Table 4). This outcome supports our hypothesis that CSSO supplementation enhances the IFNt-signaling cascade (Spencer and Bazer, 2004) and increases pregnancy success as in Exp. 1, corroborating with similar outcomes in *B. indicus* cattle consuming tropical feed ingredients (Lopes et al., 2009; Lopes et al., 2011; Cipriano et al., 2016). No treatment effects were detected (P = 0.30) for mRNA expression of *prostaglandin E synthase* nor mRNA expression of *cyclooxygenase-2* and *prostaglandin E synthase* in the endometrium (Table 4). Hence, CSSO supplementation to *B. taurus* beef cows increased expression of IFNt in the conceptus without modulating expression of prostaglandin-related genes in conceptus and endometrial tissues on day 15 of gestation.

Table 3. Ovarian variables in beef cows supplemented with CSSO or prilled saturated fat (CON) in Exp. 2[a]

Item	CSSO	CON	SEM	P value
Dominant follicle diameter (day 0), mm	16.6	15.7	0.44	0.14
Corpus luteum volume, cm^3	7.11	7.00	0.35	0.83
Plasma progesterone, ng/mL	4.20	4.35	0.32	0.73

[a]Treatments were offered from day 0 (timed-AI) to 21 of the experiment. Transrectal ultrasonography (7.5-MHz transducer; 500V, Aloka, Wallingford, CT) was performed on days 0, 7, and 15 of the experiment. Blood samples were collected for progesterone analysis on days 7 and 15. Results reported for corpus luteum and progesterone are means across days 7 and 15.

Table 4. Expression of genes associated with pregnancy establishment in the endometrium, conceptus, and blood from beef cows supplemented with CSSO or prilled saturated fat (CON) in Exp. 2[a]

Item	CSSO	CON	SEM	P value
Endometrium[b]				
Cyclooxygenase-2	4.88	5.11	1.32	0.89
Prostaglandin E synthase	5.76	7.40	1.10	0.30
Conceptus[2]				
IFNt	21.3	12.1	3.4	0.05
Prostaglandin E synthase	2.22	2.50	0.48	0.69
Blood cells[c]				
Interferon-stimulated gene 15	43.1	29.8	4.6	0.04
Myxovirus resistance 2	20.2	20.1	2.7	0.98
20,50-oligoadenylate synthetase	26.8	18.3	2.7	0.03

[a]Treatments were offered from day 0 (timed-AI) to 21 of the experiment. Values are expressed as relative fold change compared to threshold cycle of reference genes analyzed within the same sample (Cipriano et al., 2016).

[b]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.

[c]Blood samples were collected from nonflushed cows (CSSO, n = 25; CON, n = 21) on day 20 of the experiment whole-blood RNA extraction. Only samples from cows diagnosed as pregnant on day 30 were analyzed.

Synthesis of IFNt by the conceptus upregulates mRNA expression of interferon-stimulated genes (**ISG**) in circulating blood leukocytes. For this reason, mRNA expression of ISGs in whole blood has been used evaluate IFNt production and conceptus development from days 15 to 22 of gestation (Cipriano et al., 2016). Accordingly, blood mRNA expression of the ISGs *interferon-stimulated gene 15* and *20,50-oligoadenylate synthetase* on day 20 of the experiment was greater ($P \leq 0.04$)

for CSSO-supplemented compared with CON cows diagnosed as pregnant. These outcomes provide further support that CSSO supplementation enhanced IFNt synthesis by the conceptus during the pregnancy recognition period (Spencer and Bazer, 2004) in *B. taurus* beef cows.

IMPLICATIONS

Post-AI CSSO supplementation to *B. taurus* beef cows improved pregnancy rates to timed-AI (Exp. 1), which can be associated with increased mRNA expression of IFNt by the conceptus (Exp. 2) when CSSO is supplemented during early gestation. Collectively, research from our group provides evidence that CSSO supplementation after 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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