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Strategic supplementation of calcium salts of polyunsaturated fatty acids to enhance reproductive performance of *Bos indicus* beef cows

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ABSTRACT: Five experiments evaluated the effects of supplemental Ca salts of PUFA on reproductive function of *Bos indicus* beef cows. In Exp. 1, nonlactating and multiparous grazing cows (n = 51) were assigned to receive (as-fed basis) 0.1 kg of a protein-mineral mix + 0.1 kg of ground corn per cow/d, in addition to 0.1 kg per cow/d of 1) Ca salts of PUFA (PF), 2) Ca salts of SFA (SF), or 3) kaolin (control). Treatments were offered from d 0 to 20 of the estrous cycle. No treatment effects were detected on serum progesterone concentrations ($P = 0.83$), day of luteolysis ($P = 0.86$), or incidence of short cycles ($P = 0.84$). In Exp. 2, nonlactating and multiparous grazing cows (n = 43) were assigned to receive PF, SF, or control from d 0 to 8 of the estrous cycle. On d 6, all cows received (intramuscularly) 25 mg of $PGF_{2\alpha}$. No treatment effects were detected on serum progesterone concentrations on d 6 ($P = 0.37$), and incidence ($P = 0.67$) or estimated time of luteolysis ($P = 0.44$). In Exp. 3, twenty-seven lactating and multiparous grazing cows, approximately 30 to 40 d postpartum, were assigned to receive PF or control for 10 d beginning at the first postpartum ovulation. No treatment effects were detected ($P = 0.85$) on incidence of short cycles. In Exp. 4, lactating and multiparous grazing cows (n = 1,454), approximately 40 to 60 d postpartum, were assigned to receive 1 of the 7 treatments for 28 d after timed AI (**TAI**; d 0): 1) control from d 0 to 28, 2) SF from d 0 to 14 and then control, 3) PF from d 0 to 14 and then control, 4) SF from d 0 to 21 and then control, 5) PF from d 0 to 21 and then control, 6) SF from d 0 to 28, and 7) PF from d 0 to 28. Cows receiving PF for more than 21 d after TAI had greater ($P < 0.01$) pregnancy to TAI compared with all other treatments combined (50.4 vs. 42.4%, respectively). In Exp. 5, lactating and multiparous grazing cows (n = 501), approximately 40 to 60 d postpartum, were assigned to receive 1 of the 4 treatments for 21 d after TAI (d 0): 1) PF from d 0 to 14 and then control, 2) control from d 0 to 6 and then PF, 3) control from d 0 to 13 and then PF, and 4) PF from d 0 to 21. Cows receiving PF after d 14 of the experiment had greater ($P = 0.02$) pregnancy to TAI compared with cows not receiving PF during the same period (46.8 vs. 33.1%, respectively). In summary, supplemental Ca salts of PUFA during the expected time of luteolysis increased pregnancy to TAI in beef cows.

Key words: beef cow, luteolysis, polyunsaturated fatty acid, pregnancy, progesterone

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INTRODUCTION

The major objective of cow-calf systems is to produce 1 calf per cow annually. Therefore, reproductive performance of the cowherd determines the overall efficiency of cow-calf operations. Recent studies from our research group demonstrated that supplementation of Ca salts of PUFA after breeding was beneficial to reproductive performance of beef females (Lopes et al., 2009). More specifically, PUFA supplementation during the period when luteolysis would occur (Figueiredo et al., 1997) increased pregnancy rates in Nelore (*Bos indicus*) beef cows (Lopes et al., 2009). Consequently, strategic postbreeding PUFA supplementation can be adopted by cow-calf producers as a nutritional alternative to enhance reproductive performance of the cowherd.

Beneficial postbreeding effects of PUFA include modulation of $PGF_{2\alpha}$ synthesis and luteolysis (Williams and Stanko, 2000; Funston, 2004), increased circulating progesterone concentrations (Grummer and Carroll, 1991; Lopes et al., 2009), and enhanced maternal recognition of pregnancy (Wathes et al., 2007). However, additional research is required to better understand the physiological mechanisms associating PUFA and early pregnancy maintenance in beef cows. This resultant

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knowledge will promote the development and improvement of strategic PUFA supplementation programs to enhance reproductive efficiency of beef females.

Five experiments were conducted to investigate the effects of postbreeding PUFA supplementation on reproductive function of *B. indicus* females. Experiment 1 evaluated if PUFA influences circulating progesterone concentrations during the estrous cycle and timing of luteolysis. Experiment 2 evaluated if PUFA alters the sensitivity of a 6-d corpus luteum to exogenous $PGF_{2\alpha}$ treatment. Experiment 3 evaluated if PUFA alters the incidence of short cycles, whereas Exp. 4 and 5 compared pregnancy to timed AI of beef cows receiving postbreeding PUFA supplementation in different lengths.

MATERIALS AND METHODS

The animals utilized herein were cared for in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Animals, Treatments, and Sampling

Exp. 1. This experiment was conducted from October to November 2008 at a commercial cow-calf operation located in São Paulo, Brazil. Sixty nonlactating multiparous Nelore (*B. indicus*) cows (BCS = 4.8 ± 0.03; Wagner et al., 1988) were assigned to an estrous synchronization protocol (Meneghetti et al., 2009) from d −11 to 0. At the end of the estrous synchronization protocol (d 0), cows were stratified by BCS and allocated to 12 *Brachiaria brizantha* pastures (5 cows/pasture) with similar and adequate forage availability. Pastures were randomly assigned to receive (as-fed basis) 0.1 kg of a protein-mineral mix + 0.1 kg of ground corn per cow daily, in addition to 0.1 kg/cow daily of Ca salts of PUFA source (**PF**; Megalac-E, Quimica Geral do Nordeste, Rio de Janeiro, Brazil), or 0.1 kg/cow daily of Ca salts of SFA source (**SF**; Megalac, Church and Dwight, Princeton, NJ), or 0.1 kg/cow daily of kaolin (control; rumen-inert indigestible substance). Pasture was considered the experiment unit (n = 4/treatment). Treatments were offered daily at 0800 h, from d 0 to 20 of the experiment, and bunk space was approximately 1.0 m/cow. The PF and SF treatments were isocaloric and isonitrogenous, but differed in fatty acid (**FA**) profile. Composition and nutritional content of treatments are described in Tables 1 and 2. Blood samples were collected on d 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, and 20, approximately 8 to 10 h after supplements were offered for determination of serum progesterone concentrations. Luteolysis was determined when serum progesterone concentrations were <1.5 ng/mL. This criterion was adopted herein and in the subsequent experiments because of potential contribution of adrenal progesterone to the total circulating progesterone in *B. indicus*-influenced females (Cooke and Arthington, 2009). Cows with serum progesterone concentrations <1.5 ng/mL on d 2, but progesterone concentrations increasing and ≥1.5 ng/mL on d 4 and 6, were classified as ovulated. Only samples from ovulated cows were used in the subsequent analysis (n = 51). Short cycles were determined in ovulated cows that experienced luteolysis before d 10 of the experiment.

Table 1. Composition and nutrient profile of protein-mineral mixes offered during the study

Item	Exp. 1 and 2[1]	Exp. 3 to 5[2]
Ingredient, as-fed basis		
Cane molasses, %	—	2.50
Ground corn, %	—	26.00
Citrus pulp, %	2.00	—
Soybean meal, %	—	20.00
Cottonseed meal, %	42.00	—
Urea, %	4.50	6.25
Calcium diphosphate, %	—	17.50
Calcium carbonate, %	—	2.50
Elemental S, %	—	0.25
Mineral mix, %	51.5[3]	5.00[4]
Sodium chloride, %	—	20.00
Nutrient profile, DM basis		
DM, %	93.0	94.1
TDN, %	44.0	38.0
CP, %	30.0	31.6
NDF, %	2.9	4.2
Ether extract, %	0.1	1.2
Ca, %	7.7	5.2
P, %	2.0	3.8

[1]Values provided by manufacturer (Lambisk V, Bellman Nutrição Animal Ltda., Mirassol, São Paulo, Brazil).

[2]Values provided by manufacturer (Propec Consultoria Rural Ltda., Campo Grande, Mato Grosso do Sul, Brazil).

[3]Values not provided by manufacturer.

[4]Contained 12.0% Mg, 16.5% S, 0.10% Co, 1.25% Cu, 0.08% I, 0.96% Mn, 0.01% Se, 2.8% Zn, 1.1% Fe.

Exp. 2. This experiment was conducted from November to December 2008 at a commercial cow-calf operation located in São Paulo, Brazil. Fifty-three nonlactating multiparous Nelore cows (BCS = 4.9 ± 0.03;

Table 2. Fatty acid profile of fatty acid sources (Ca salts) offered during the study[1]

Item	PUFA[2]	SFA[3]
Lauric acid (12:0), %	0.1	0.2
Mystiric acid (14:0), %	0.2	1.6
Palmitic acid (16:0), %	17.5	50.8
Palmitoleic acid (16:1), %	0.3	0.0
Stearic acid (18:0), %	5.1	4.1
Oleic acid (18:1), %	31.7	35.7
Linoleic acid (18:2), %	39.8	7.0
Linolenic acid (18:3), %	2.7	0.2
Other	2.6	0.4

[1]As percentage of total fatty acids. Values provided by manufacturers.

[2]Megalac-E (Quimica Geral do Nordeste, Rio de Janeiro, Brazil), derived from soybean oil.

[3]Megalac (Church & Dwight Co. Inc., Princeton, NJ), derived from palm oil.

Wagner et al., 1988) were assigned to an estrous synchronization protocol (Meneghetti et al., 2009) from d −11 to 0. At the end of the estrous synchronization protocol (d 0), cows were stratified by BCS and allocated to 12 *B. brizantha* pastures (4 or 5 cows/pasture) with similar and adequate forage availability. Pastures were randomly assigned to receive PF, SF, or control. Pasture was considered the experiment unit (n = 4/treatment). Treatments were offered daily at 0800 h, from d 0 to 8 of the experiment, and bunk space was approximately 1.0 m/cow. Composition and nutritional profile of treatments are described in Tables 1 and 2. On d 6 of the experiment, all cows received a $PGF_{2\alpha}$ treatment (25 mg of Lutalyse, Pfizer Animal Health, São Paulo, Brazil) to evaluate treatment effects on luteolysis. Blood samples were collected on d 3 and 6 (immediately before to $PGF_{2\alpha}$ treatment), and every 12 h from d 6 to 8 for determination of serum progesterone concentrations. Cows with serum progesterone concentrations <1.5 ng/mL on d 3, but progesterone concentrations ≥1.5 ng/mL on d 6, were classified as ovulated. Only samples from ovulated cows were used in the subsequent analysis (n = 43). Luteolysis was determined when serum progesterone concentrations from samples collected after the $PGF_{2\alpha}$ treatment were <1.5 ng/mL (Cooke and Arthington, 2009). Cows with serum progesterone concentrations ≥1.5 ng/mL 48 h after $PGF_{2\alpha}$ treatment were considered as having a functional corpus luteum and not responsive to the $PGF_{2\alpha}$ treatment.

Exp. 3. This experiment was conducted in April 2009 at a commercial cow-calf operation located in Mato Grosso do Sul, Brazil. Forty-six anestrous, lactating multiparous Nelore cows (BCS = 4.8 ± 0.02; Wagner et al., 1988), approximately 30 to 40 d postpartum, were assigned to an estrous synchronization protocol, consisting of 48-h calf removal (d −2) and a 100-μg treatment of GnRH (Fertagyl, Intervet/Schering-Plough Animal Health, São Paulo, Brazil) on d 0 concurrent with calf return. Estrous cyclicity was evaluated by detecting the presence of a corpus luteum, via transrectal ultrasonography (7.5-MHz transducer, 500V, Aloka, Wallingford, CT) on d −14, 0, and 2. Cows without a corpus luteum on d −14 and 0 were classified as anestrous. At the end of the estrous synchronization protocol (d 0), cows were stratified by BCS and allocated to 8 *B. brizantha* pastures (5 or 6 cows/pasture) with similar and adequate forage availability. Pastures were randomly assigned to receive PF or control. Pasture was considered the experiment unit (n = 4/treatment). Treatments were offered daily at 0800 h, from d 0 to 10 of the experiment, and bunk space was approximately 0.8 m/cow. Composition and nutritional profile of treatments are described in Tables 1 and 2. Ovulation was verified by disappearance of the largest follicle from d 0 to 2 via transrectal ultrasonography (7.5-MHz transducer, 500V, Aloka). Only cows classified as ovulated were maintained in the experiment (n = 27). No experimental units were lost when cows that did not ovulate were removed from the experiment, and number of cows per pasture ranged from 2 to 4. Blood samples were collected on d 7, 8, 9, and 10, approximately 3 to 4 h after supplements were offered for determination of serum progesterone concentrations. Luteolysis was determined when serum progesterone concentrations were <1.5 ng/mL (Cooke and Arthington, 2009). Short cycles were determined in ovulated cows that experienced luteolysis before d 10 of the experiment.

Exp. 4. This experiment was conducted in January and February 2009 at a commercial cow-calf operation located in Mato Grosso do Sul, Brazil. A total of 1,454 lactating multiparous Nelore cows (BCS = 4.7 ± 0.05), approximately 40 to 60 d postpartum, were assigned to the estrous synchronization protocol described by Meneghetti et al. (2009) from d −11 to 0, with the inclusion of calf removal from d −2 to 0. All cows were assigned to fixed-time AI on d 0. To account for potential bull and technician effects, semen source and AI technicians were equally distributed among pastures and treatments. Immediately after AI (d 0), cows were allocated randomly to 28 *Brachiaria humidicula* pastures (approximately 52 cows/pasture) with similar and adequate forage availability. Pastures were considered the experimental unit, and assigned randomly to 1 of the 7 treatment combinations: 1) control from d 0 to 28 (n = 3 pastures), 2) SF from d 0 to 14 and control from d 15 to 28 (**SF14**; n = 3 pastures), 3) PF from d 0 to 14 and control from d 15 to 28 (**PF14**; n = 6 pastures), 4) SF from d 0 to 21 and control from d 22 to 28 (**SF21**; 3 pastures), 5) PF from d 0 to 21 and control from d 22 to 28 (**PF21**; 5 pastures), 6) SF from d 0 to 28 (**SF28**; 3 pastures), and 7) PF from d 0 to 28 (**PF28**; 5 pastures). Treatments were not uniformly distributed across pastures because of reduced availability of the SFA source and to comply with the management scheme of the operation where the experiment was conducted, which adopted postbreeding inclusion of PUFA because of the benefits described previously by our research group (Lopes et al., 2009). Treatments were offered daily at 0800 h, and bunk space was approximately 1.0 m/cow. Composition and nutritional content of treatments are described in Tables 1 and 2. Pregnancy status was verified by detecting a fetus with transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka) on d 28.

Exp. 5. This experiment was conducted in February and March 2009 at a commercial cow-calf operation located in Mato Grosso, Brazil. A total of 501 lactating multiparous Nelore cows (BCS = 4.8 ± 0.04), approximately 40 to 60 d postpartum, were assigned to an estrous synchronization + timed-AI protocol as in Exp. 4. Immediately after timed AI (d 0), cows were allocated randomly to 16 *B. brizantha* pastures (approximately 31 cows/pasture) with similar and adequate forage availability. Pastures were considered the experimental unit, and assigned randomly to 1 of the 4 treatment combinations: 1) PF from d 0 to 14 and control from d 15 to 21 (**PF0–14**; n = 4 pastures), 2)

control from d 0 to 6 and PF from d 7 to 21 (**PF7–21**; n = 4 pastures), 3) control from d 0 to 13 and PF from 14 to 21 (**PF14–21**; n = 3 pastures), and 4) PF from d 0 to 21 (**PF0–21**; n = 5 pastures). As in Exp. 4, treatments were not uniformly distributed into pastures to comply with the management scheme of the operation where the experiment was conducted, which adopted postbreeding inclusion of PUFA because of the benefits described previously by our research group (Lopes et al., 2009). Treatments were offered daily at 0800 h, and bunk space was approximately 0.8 m/cow. Composition and nutritional content of treatments are described in Tables 1 and 2. Pregnancy status was verified by detecting a fetus with transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka) on d 28.

Blood Analysis

For Exp. 1, 2, and 3, blood was collected via coccygeal vein or artery into commercial blood collection tubes (10 mL, Vacutainer, Becton Dickinson, Franklin Lakes, NJ), placed on ice immediately, maintained at 4°C for 24 h, centrifuged at 3,000 × *g* for 10 min at room temperature for serum collection, and frozen at −20°C until further processing. Concentrations of progesterone were determined using a Coat-A-Count solid phase ^{125}I RIA kit (Siemens Medical Solutions Diagnostics, Dallas, TX). Samples were analyzed after all experiments were concluded. The intra- and interassay CV were 5.1 and 6.3%, respectively. The minimum detectable concentration of progesterone was 0.1 ng/mL.

Statistical Analysis

Serum progesterone concentrations (Exp. 1, 2, and 3) and timing of luteolysis (Exp. 1 and 2) were analyzed with the MIXED procedure (SAS Inst. Inc., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement for progesterone analysis contained the effects of treatment, time variable (hour or day), and the interaction. Data were analyzed using pasture(treatment) and cow(pasture) as random variables. The specified term for the repeated statement was day (Exp. 1 and 3) or hour (Exp. 2), the subject was cow(pasture), and the covariance structure used was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. When progesterone data collected on d 6 within Exp. 1 and 2 are combined and analyzed jointly, the model statement contained the effects of treatment, experiment, and the resultant interaction. Data were analyzed using pasture(treatment × experiment) and cow(pasture) as random variables. The model statement for time of luteolysis contained the effect of treatment. Data were analyzed using pasture(treatment) and cow(pasture) as random variables.

Incidence of short cycles (Exp. 3) and pregnancy to timed AI (Exp. 4 and 5) were analyzed as binary data with the GLIMMIX procedure of SAS with a binomial distribution and logit link function. Both model statements contained the effects of treatment, whereas pasture(treatment) and cow(pasture) were used as random variables.

For all analyses, significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected. Pregnancy to timed AI and incidence of short cycles are reported as means, whereas serum progesterone and timing of luteolysis are reported as least squares means. Results were separated using LSD (Exp. 3) or PDIFF (Exp. 1, 2, 4, and 5). Results from Exp. 4 and Exp. 5 were also analyzed using predetermined single-degree-of-freedom contrasts to directly evaluate PUFA supplementation during the expected time of luteolysis (d 16 after ovulation; Figueiredo et al., 1997). In Exp. 4, the following contrasts were used: 1) control + PF14 + SF14 vs. PF21 + PF28, compare cows receiving PUFA or no supplemental fat on d 16; 2) control + PF14 + SF14 vs. SF21 + SF28, compare cows receiving SFA or no supplemental fat on d 16; 3) control + SF14 + PF14 vs. SF21 + SF28 + PF21 + PF28, compare cows receiving supplemental fat or not on d 16; 4) SF21 + SF28 vs. PF21 + PF28, compare cows receiving PUFA or SFA on d 16; and 5) PF21 + PF28 vs. all other treatments, compare cows receiving PF vs. those receiving SFA and no supplemental fat on d 16. In Exp. 5, the following contrasts were used: 1) PF0–14 vs. PF0–21 + PF7–21 + PF14–21, compare cows receiving PF or no supplemental fat on d 16; 2) PF14–21 vs. PF0–21 + PF7–21, compare timing of beginning of supplementation in cows receiving PUFA on d 16; and 3) PF0–21 vs. PF7–21 + PF14–21, compare timing of beginning of supplementation in cows receiving PUFA on d 16.

RESULTS

Exp. 1

No treatment effects were detected ($P = 0.83$; Table 3) on serum progesterone concentrations during the estrous cycle (2.54, 2.62, and 2.76 ng/mL for control, SF, and PF, respectively; SEM = 0.26). No treatment effects were detected ($P = 0.86$; Table 3) on day of luteolysis (17.5, 17.0, and 17.2 for control, SF, and PF, respectively; SEM = 0.64). No treatment effects were detected ($P = 0.84$; Table 3) on incidence of short cycles (12.5, 11.7, and 5.8% for control, SF, and PF, respectively).

Exp. 2

No treatment effects were detected ($P = 0.67$; Table 3) on incidence of luteolysis after the $PGF_{2\alpha}$ treatment (57.1, 76.9, and 62.5% for control, SF, and PF, respectively). Within cows that experienced luteolysis, no

Table 3. Reproductive variables of beef cows supplemented or not with Ca salts of PUFA[1,2]

Item	Control	SF	PF	SEM	*P*-value
Exp. 1[3]					
Experimental pasture, n	4	4	4	—	—
Serum progesterone, ng/mL	2.54	2.62	2.76	0.26	0.83
Day of luteolysis, d	17.5	17.0	17.2	0.64	0.86
Incidence of short cycles, %	12.5	11.7	5.8	—	0.84
Exp. 2[4]					
Experimental pasture, n	4	4	4	—	—
Incidence of luteolysis, %	57.1	76.9	62.5	—	0.67
Time of luteolysis, h	20.7	14.0	17.1	3.5	0.44
Serum progesterone on d 6, ng/mL	3.53	3.16	4.15	0.47	0.37
Exp. 3[5]					
Experimental pasture, n	4	—	4	—	—
Incidence of short cycles, %	46.1	—	50.0	—	0.85

[1]Cows were offered (as-fed basis) 0.1 kg of a protein-mineral mix + 0.1 kg of ground corn per cow daily in addition to 0.1 kg/cow daily of Ca salts of PUFA (PF; Megalac-E, Quimica Geral do Nordeste, Rio de Janeiro, Brazil), 0.1 kg/cow daily of Ca salts of SFA (SF; Megalac, Church and Dwight, Princeton, NJ), or 0.1 kg/cow daily of kaolin (control; rumen-inert indigestible substance).

[2]Luteolysis was determined when serum progesterone concentrations were <1.5 ng/mL. Short cycles were determined in ovulated cows that experienced luteolysis before d 10 of the experiment.

[3]Treatments were offered from d 0 to 20 of the estrous cycle. Blood samples were collected on d 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, and 20.

[4]Treatments were offered from d 0 to 8 of the estrous cycle. On d 6 of the experiment, all cows received a $PGF_{2\alpha}$ treatment to stimulate luteolysis. Blood samples were collected every 12 h from d 6 to 8.

[5]Treatments were offered from d 0 to 10 of the estrous cycle. Blood samples were collected on d 7, 8, 9, and 10.

treatment effects were detected ($P = 0.44$; Table 3) on estimated time of luteolysis after the $PGF_{2\alpha}$ treatment (20.7, 14.0, and 17.1 h for control, SF, and PF, respectively; SEM = 3.5).

No treatment effects were detected ($P = 0.37$; Table 3) on serum progesterone concentrations on d 6 before the $PGF_{2\alpha}$ treatment (3.53, 3.16, and 4.15 ng/mL for control, SF, and PF, respectively; SEM = 0.47). However, when samples collected on d 6 from Exp. 1 and 2 are combined and analyzed jointly; a treatment effect was detected ($P = 0.01$; Figure 1) because PF cows had greater progesterone concentrations compared with control ($P = 0.02$) and SF ($P < 0.01$) cows (4.45, 3.48, and 3.25 ng/mL, respectively; SEM = 0.28).

Exp. 3 and 4

In Exp. 3, no treatment effects were detected ($P = 0.85$; Table 3) on incidence of short cycles during the first postpartum estrous cycle (46.1 and 50.0% for control and PF, respectively). In Exp. 4, a treatment effect was not detected for pregnancy to timed AI ($P = 0.22$; Table 4) given that no significant differences were detected ($P \geq 0.07$) when treatments were individually compared with each other. However, cows receiving PF at d 16 relative to TAI (PF21 + PF28; 50.3% of pregnant cows/total cows) had greater ($P = 0.03$) pregnancy to timed AI compared with cohorts concurrently receiving SF (SF21 + SF28; 41.9% of pregnant cows/total cows) or control ($P = 0.01$; control + SF14 + PF14; 42.4% of pregnant cows/total cows), as well as compared with all other treatments combined ($P < 0.01$; control + SF14 + SF21 + SF28 + PF14; 42.2% of pregnant cows/total cows). Further, pregnancy to timed AI was similar ($P = 0.79$) between PF21 and PF28 (51.0 vs. 49.8% of pregnant cows/total cows, respectively).

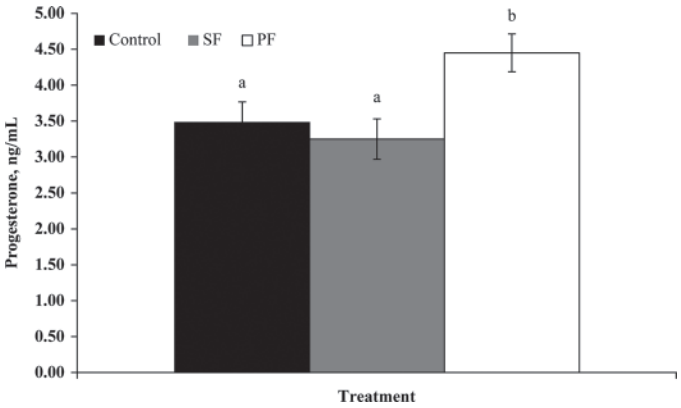

Figure 1. Serum progesterone concentrations on d 6 of the estrous cycle in nonlactating and nonpregnant multiparous beef cows maintained on pasture and receiving supplements (0.1 kg of a protein-mineral mix + 0.1 kg of ground corn per cow daily; as-fed basis) in addition to 0.1 kg/cow daily of Ca salts of PUFA (PF; Megalac-E, Quimica Geral do Nordeste, Rio de Janeiro, Brazil), 0.1 kg/cow daily of Ca salts of SFA (SF; Megalac, Church and Dwight, Princeton, NJ), or 0.1 kg/cow daily of kaolin (control; rumen-inert indigestible substance). Treatments were offered daily, beginning on d 0 of the estrous cycle. A treatment effect was detected ($P = 0.01$). Values with a different letter (a,b) differ at $P \leq 0.02$.

Table 4. Pregnancy to timed AI (pregnant cows/total inseminated cows) of beef cows supplemented or not with Ca salts of PUFA after timed AI

Item[1]	Pregnancy to timed AI,[2] %	Main effect *P*-value	Contrast[3,4] 1	2	3	4	5
Exp. 4[5]							
Control	41.6 (65/156)	0.22	0.01	0.95	0.18	0.03	<0.01
SF14	41.9 (67/160)						
SF21	42.7 (67/157)						
SF28	41.3 (64/155)						
PF14	43.1 (131/304)						
PF21	51.0 (128/251)						
PF28	49.8 (135/271)						
Exp. 5[6]							
PF0–14	33.1 (42/127)[a]	0.05	0.02	0.42	0.26	—	—
PF0–21	50.3 (78/155)[b]						
PF7–21	45.3 (58/128)[ab]						
PF14–21	42.8 (39/91)[ab]						

[a,b]Values with different superscripts differ ($P < 0.05$).

[1]Treatments consisted of 0.1 kg of a protein-mineral mix + 0.1 kg of ground corn per cow daily in addition to 0.1 kg/cow daily of Ca salts of PUFA (PF; Megalac-E, Quimica Geral do Nordeste, Rio de Janeiro, Brazil), 0.1 kg/cow daily of Ca salts of SFA (SF; Megalac, Church and Dwight, Princeton, NJ), or 0.1 kg/cow daily of kaolin (control; rumen-inert indigestible substance).

[2]Pregnancy to timed AI is reported as means. Values in parentheses represent number of pregnant cows/total cows.

[3]Contrasts for Exp. 4: 1) control + PF14 + SF14 vs. PF21 + PF28, 2) control + PF14 + SF14 vs. SF21 + SF28, 3) control + SF14 + PF14 vs. SF21 + SF28 + PF21 + PF28, 4) SF21 + SF28 vs. PF21 + PF28, and 5) PF21 + PF28 vs. all other treatments.

[4]Contrasts for Exp. 5: 1) PF0–14 vs. PF0–21 + PF7–21 + PF14–21, 2) PF14–21 vs. PF0–21 + PF7–21, and 3) PF0–21 vs. PF7–21 + PF14–21.

[5]Control = control treatment from d 0 to 28 after AI (n = 3 pastures); SF14 = SF from d 0 to 14 and control from d 15 to 28 (n = 3 pastures); SF21 = SF from d 0 to 21 and control from d 22 to 28 (3 pastures); SF28 = SF from d 0 to 28 (3 pastures); PF14 = PF from d 0 to 14 and control from d 15 to 28 (n = 6 pastures); PF21 = PF from d 0 to 21 and control from d 22 to 28 (5 pastures); PF28 = PF from d 0 to 28 (5 pastures).

[6]PF0–14 = PF from d 0 to 14 and control from d 15 to 21 (n = 4 pastures); PF0–21 = PF from d 0 to 21 (n = 5 pastures); PF7–21 = control from d 0 to 6 and PF from d 7 to 21 (n = 4 pastures); PF14–21 = control from d 0 to 13 and PF from 14 to 21 (n = 3 pastures).

Exp. 5

A treatment effect was detected for pregnancy to timed AI ($P = 0.05$; Table 4). Cows receiving PF0–21 had greater ($P = 0.01$) pregnancy to timed AI compared with PF0–14 (50.3 vs. 33.1% of pregnant cows/total cows, respectively). Cows receiving PF7–21 tended to have greater ($P = 0.07$) pregnancy to timed AI compared with PF0–14 (45.3% of pregnant cows/total cows for PF7–21). Furthermore, cows receiving PF during d 16 after ovulation (PF0–21 + PF7–21 + PF14–21; 46.8% of pregnant cows/total cows) had greater pregnancy to timed AI ($P = 0.02$) compared with cows not receiving PF during the same period (PF0–14). Pregnancy to timed AI was similar between PF0–21, PF7–21, and PF14–21 ($P \geq 0.26$).

DISCUSSION

In the present study, Ca salts of PUFA improved pregnancy to timed AI in beef cows only when supplemented during the expected time of luteolysis (around d 16 after ovulation in *B. indicus* females; Figueiredo et al., 1997). These results support previous research from our group that, to our knowledge, first demonstrated the beneficial effects of postbreeding PUFA supplementation on reproductive performance of beef cows (Lopes et al., 2009). More specifically, as reported in Exp. 4, PUFA supplementation is required for at least 21 d after AI to elicit beneficial effects on pregnancy to timed AI. In the same experiment, extending PUFA supplementation to 28 d after AI did not yield any additional benefits on reproductive performance of beef cows. Mann and Lamming (2001) reported that 85% of serviced cows had a developing embryo within uterine material collected on d 16 after breeding. The same authors, however, reported that almost 50% of these cows returned to estrus by d 21 after breeding, indicating that significant embryo losses occur during the expected time of luteolysis. Therefore, postbreeding PUFA supplementation likely increased pregnancy to timed AI herein and in Lopes et al. (2009) by alleviating pregnancy losses that occur around d 16 after conception and later. Further, the beneficial effects of PUFA on pregnancy to timed AI were independent of its contribution to nutrient intake, given that the PF and SF treatments were based on Ca salts of FA, isocaloric, isonitrogenous, and isolipidic, and cows receiving

the SF treatment had similar pregnancy to timed AI compared with control cows.

Results from Exp. 5 indicate that strategic PUFA supplementation regimens can be adopted to benefit pregnancy rates of beef cows. An experimental group receiving control or SFA from d 0 to 21 was not included in Exp. 5 given that, based on Exp. 4 and data from Lopes et al. (2009), supplementation of Ca salts of PUFA for less than 16 d after AI yields similar pregnancy rates compared with cows receiving control or SFA for 28 d after AI. Still, the additional energy provided by 0.1 kg of the Ca salts of PUFA source accounted for only 2.8% of the daily TDN requirement of cows in Exp. 5 (NRC, 1996), which can be considered marginal and insufficient to contribute to the differences in pregnancy to timed AI detected in Exp. 5. Similar to Exp. 4, PUFA supplementation during the expected time of luteolysis, independent of when supplementation started, had beneficial effects on pregnancy to timed AI (PF0–14 vs. PF0–21 + PF7–21 + PF14–21). However, the most significant improvement was detected when cows received PUFA for 21 d after breeding (PF0–14 vs. PF0–21). Continuous feeding of FA sources is required to manipulate the FA content of body tissues in cattle, including embryonic and tissues within the reproductive tract (Mattos et al., 2000; Bilby et al., 2006). Therefore, cows receiving PF beginning on d 7 and 14 after AI likely did not have sufficient time to incorporate adequate amounts of PUFA into tissues compared with cows receiving PUFA beginning after AI. Nevertheless, circulating FA profile and FA incorporation into maternal and embryonic tissues were not evaluated in the present experiment. Such assessments are warranted to further understand how postbreeding PUFA supplementation benefits reproduction in beef females.

The mechanisms by which postbreeding PUFA supplementation enhances pregnancy rates in beef cows include increased circulating progesterone concentrations (Stronge et al., 2005; Demetrio et al., 2007; Lopes et al., 2009), modulation of $PGF_{2\alpha}$ synthesis and luteolysis (Williams and Stanko, 2000; Funston, 2004), and enhanced maternal recognition of pregnancy (Wathes et al., 2007). More specifically, progesterone prepares the uterine environment for conceptus growth and development, controls endometrial secretions and structural changes that are essential for proper embryo development (Gray et al., 2001; Wang et al., 2007), modulates the release of hormones that may regress the corpus luteum and disrupt gestation (Bazer et al., 1998), and is required for proper establishment of pregnancy (Spencer and Bazer, 2002). Supplementation with PUFA increases circulating progesterone concentrations by increasing circulating cholesterol availability, the major precursor for luteal progesterone synthesis (Grummer and Carroll, 1991; Son et al., 1996), as well as alleviating hepatic steroid metabolism (Hawkins et al., 1995; Sangsritavong et al., 2002; Lopes et al., 2009). However, no treatment effects were detected in serum progesterone concentrations in Exp. 1 when samples were collected during the estrous cycle. No treatment effects were also detected in serum progesterone concentrations from samples collected on d 6 within Exp. 2, before $PGF_{2\alpha}$ treatment. Nevertheless, when samples collected on d 6 within Exp. 1 and 2 are combined and analyzed jointly, given that supplements, FA sources, pastures, and locations were similar between Exp. 1 and 2, cows receiving PF had greater serum progesterone concentrations compared with SF and control cows. Other authors also reported increased circulating progesterone concentrations in cows receiving supplemental PUFA (Talavera et al., 1985; Hawkins et al., 1995; Staples et al., 1998), which is expected to benefit pregnancy establishment and maintenance. However, increased progesterone concentrations only on d 6 after AI might not be sufficient to explain the reproductive benefits of postbreeding PUFA supplementation, although our group reported a positive association among conception rates and serum progesterone concentrations on d 7 after AI in dairy cows (Demetrio et al., 2007). Accordingly, cows receiving PF from d 7 to 21 after TAI had improved pregnancy to timed AI compared with cohorts receiving PF from d 0 to 14 after TAI in Exp. 5, indicating that the reproductive benefits of PUFA were independent of its contribution to increased circulating progesterone during the initial 7 d after TAI.

Postbreeding PUFA supplementation may also enhance circulating progesterone concentrations and overall pregnancy rates by modulating uterine synthesis of $PGF_{2\alpha}$ and consequently corpus luteum lifespan (Lucy et al., 1991; Mattos et al., 2002). In fact, Williams and Stanko (1999) indicated that one of the main mechanisms by which PUFA enhances pregnancy establishment and maintenance is by delaying luteolysis. Burke et al. (1997) reported that PUFA decreases the overall responsiveness of the corpus luteum to $PGF_{2\alpha}$, whereas Oldick et al. (1997) reported that PUFA infusion delayed luteolysis in approximately 1 d in cows receiving exogenous $PGF_{2\alpha}$ administration on d 15 of the estrous cycle. Other researchers also demonstrated that PUFA supplementation promotes synthesis of antiluteolytic substances, including PGE_3 (Petit and Twagiramungu, 2006; Wathes et al., 2007). However, in the present study, timing of natural luteolysis (Exp. 1), responsiveness of the corpus luteum to exogenous $PGF_{2\alpha}$ administration on d 6 of the estrous cycle (Exp. 2), and incidence of short cycles (Exp. 1 and 3) were not altered by PUFA supplementation. These outcomes do not help in elucidating the means by which PUFA supplementation during the expected time of luteolysis increases pregnancy to timed AI, as described in Exp. 4 and 5 and by Lopes et al. (2009). Nevertheless, in Exp. 1, 2, and 3, the effects of PUFA supplementation on corpus luteum survival and lifespan were evaluated in nonpregnant cows. Pregnancy establishment is a complex process that involves communication between

the conceptus and maternal tissues to prevent luteolysis and consequent pregnancy loss (Spencer and Bazer, 2004). One can speculate that the conceptus is required to elicit the antiluteolytic effects of the PUFA supplement offered herein, resulting in increased pregnancy to timed AI as detected in Exp. 4 and 5. In fact, PUFA supplementation enhanced initial development of human (Haggarty et al., 2006) and swine embryos (Kojima et al., 1997). Conversely, PUFA supplementation to donor beef cows did not benefit subsequent 72-h in vitro development of recovered, cryopreserved embryos (Guardieiro et al., 2009). Therefore, adequate incorporation of PUFA into conceptus and maternal tissues, particularly during the expected time of luteolysis, might be essential to enhance pregnancy maintenance and overall reproductive performance of beef cows.

The Ca salts of PUFA source offered herein and by Lopes et al. (2009) contained greater amounts and likely resulted in increased duodenal absorption of linoleic acid compared with linolenic acid (Lopes et al., 2009). Linoleic acid can be desaturated and elongated to arachadonic acid, and serve as a precursor for $PGF_{2\alpha}$ synthesis (Yaqoob and Calder, 2007). However, previous research indicated that linoleic acid can reduce $PGF_{2\alpha}$ synthesis by inhibiting the enzymes Δ_6-desaturase and cyclooxygenase (Staples et al., 1998; Cheng et al., 2001). Nevertheless, research studies demonstrated that supplementation with linoleic acid to beef cows after breeding increased circulating concentrations of $PGF_{2\alpha}$ metabolite and reduced overall pregnancy rates (Filley et al., 2000; Grant et al., 2005). Conversely, linolenic acid is a precursor of eicosapentaenoic and docosahexaenoic acids, which have been shown to inhibit cyclooxygenase activity and consequently $PGF_{2\alpha}$ synthesis (Yaqoob and Calder, 2007). Previous research demonstrated that supplementation with linolenic acid inhibits synthesis of $PGF_{2\alpha}$, delays luteolysis, and reduces pregnancy losses in cattle (Burke et al., 1997; Thatcher et al., 1997; Mattos et al., 2000). Given that the amount and type of FA reaching target tissues dictates whether luteolysis is stimulated or inhibited (Thatcher and Staples, 2000), it cannot be concluded if PUFA supplementation benefited reproductive performance of beef cows because of the increased supply of linoleic acid, linolenic acid, or both. Consequently, further research is warranted to determine the dietary quantity and ratio of linoleic and linolenic acids required to effectively benefit reproductive function in beef females.

In conclusion, postbreeding supplementation of Ca salts of PUFA, particularly during the expected time of luteolysis, increased pregnancy to timed AI in beef cows. The mechanisms by which postbreeding PUFA supplementation benefits reproductive performance in beef females, including modulation of luteolysis, still need to be investigated further. Nevertheless, supplementing Ca salts of PUFA for 21 d beginning at AI might be a feasible alternative to enhance reproductive performance of beef cows and promote overall efficiency in cow-calf operations.

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