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Effects of rumen-protected polyunsaturated fatty acid supplementation on reproductive performance of *Bos indicus* beef cows[1]

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ABSTRACT: Five experiments evaluated the effects of rumen-protected PUFA supplementation on reproductive function of *Bos indicus* beef cows. In Exp. 1, 910 lactating primiparous Nelore cows were randomly assigned to receive 0.4 kg/d of a protein-mineral mix in addition to 0.1 kg/d of a rumen-inert PUFA source (PF) or 0.1 kg/d of kaolin (rumen-inert indigestible substance; control), from the beginning of estrus synchronization protocol (d −11) until 28 d after fixed-time AI (TAI; d 28). Cows supplemented with PF had greater ($P = 0.04$) pregnancy rates compared with control cows (51.2 vs. 39.6%). In Exp. 2, 818 lactating primiparous Nelore cows were assigned to the same TAI schedule from Exp. 1 and randomly allocated to receive 1) control from d −11 to 28, 2) PF from d −11 to 16 and control from d 17 to 28, or 3) PF from d −11 to 28. Cows receiving PF until d 28 had greater ($P = 0.02$) pregnancy rates compared with control cows and tended to have greater ($P = 0.10$) pregnancy rates compared with cows receiving PF until d 16 (42.9, 31.3, and 35.8%, respectively). In Exp. 3, 435 nulliparous and multiparous lactating *B. indicus*-crossbred cows were randomly assigned to receive control or PF from the end of synchronization protocol (d 0) until 21 d after fixed-time embryo transfer (d 28). Cows supplemented with PF had greater ($P = 0.07$) pregnancy rates compared with control cows (49.6 vs. 37.7%). In Exp. 4, 504 lactating multiparous Nelore cows were randomly assigned to receive PF or a similar supplement containing a rumen-protected SFA source (SF) for 28 d beginning after TAI. Cows supplemented with PF had greater ($P = 0.02$) pregnancy rates compared with SF cows (47.9 vs. 35.5%). In Exp. 5, 9 nonlactating, nonpregnant, ovariectomized Gir × Holstein cows inserted with an intravaginal progesterone (P_4)-releasing device were stratified by BW and BCS and divided into 3 squares. Squares were randomly assigned to receive control, PF, or a protein-mineral mix containing 0.2 kg/d of rumen-inert PUFA source (PF2), in a Latin square 3 × 3 design containing 3 periods of 14 d each. Cows supplemented with PF had greater ($P = 0.03$) mean serum P_4 concentrations compared with control and PF2 cows (1.81, 1.66, and 1.68 ng/mL, respectively). These results indicate that supplementing 0.1 kg/d of rumen-inert PUFA to beef cows, particularly after breeding, may be a method of enhancing their reproductive performance, perhaps by increasing circulating P_4 concentrations.

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

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INTRODUCTION

The major objective of cow-calf enterprises is to produce 1 calf per cow annually. Thus, management strategies that enhance reproductive performance of beef cows are beneficial to the productivity of cow-calf operations. Previous studies reported that utilization of dietary fat as a nutraceutical, particularly PUFA, positively influenced reproductive function in beef cows (Williams and Stanko, 2000). Furthermore, these positive effects were independent of the additional energy contribution from the PUFA sources (Funston, 2004).

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Strategies that increase progesterone ($\mathbf{P_4}$) concentrations in cattle before or after breeding have been positively associated with pregnancy rates (Robinson et al., 1989; Folman et al., 1990), given that P_4 is required for proper establishment and maintenance of pregnancy (Spencer and Bazer, 2002). Supplemental PUFA has been shown to increase P_4 concentrations by enhancing development of luteal cells (Lucy et al., 1991), reducing uterine synthesis of $PGF_{2\alpha}$ (Mattos et al., 2002), delaying luteolysis (Williams, 1989), and directly alleviating hepatic steroid metabolism (Sangsritavong et al., 2002). Additionally, supplemental PUFA may also increase circulating insulin concentrations, which in turn has also been shown to reduce hepatic expression of P_4 catabolic enzymes (Lemley et al., 2008).

However, PUFA originated from common feedstuffs are readily and almost completely biohydrogenated in the rumen. One alternative to increase the supply of PUFA to the small intestine is via supplementation with rumen-inert feeds such as calcium salts of fatty acids. Based on this information, we hypothesized that supplementation of rumen-protected PUFA to beef cows will enhance their reproductive performance, and this outcome can be attributed, at least in part, to increased circulating P_4 concentrations. Therefore, 5 experiments were conducted to investigate the effects of PUFA supplementation on reproductive function of *Bos indicus* females.

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).

Exp. 1

This experiment was designed to evaluate the effects of rumen-protected PUFA supplementation from the beginning of estrus synchronization protocol until 28 d after fixed-time AI (**TAI**) on pregnancy rates of brood cows and was conducted from December, 2006, to January, 2007, at 2 commercial cow-calf operations located in Mato Grosso do Sul, Brazil. Across operations, 910 lactating primiparous Nelore cows (BCS = 4.4 ± 0.03), approximately 50 to 80 d postpartum, were randomly allocated to 16 *Brachiaria decumbens* pastures (approximately 57 cows/pasture). Pastures were assigned randomly to receive (as-fed basis) 0.4 kg/cow daily of a protein-mineral mix, in addition to 0.1 kg/cow daily of a rumen-inert PUFA source (**PF**; Megalac-E, Quimica Geral do Nordeste, Rio de Janeiro, Brazil) or 0.1 kg/cow daily of kaolin (control; rumen-inert indigestible substance). Treatments were offered daily in the morning and bunk space was approximately 0.6 m/cow. Composition and nutritional profile of experimental treatments are described in Tables 1 and 2. Cows from both treatments were assigned to an estrus synchronization protocol and TAI. Treatments were offered from the beginning of protocol (d −11) until 28 d after TAI (d 28). Pregnancy status was verified by detecting a fetus with transrectal ultrasonography (Aloka SSD-500 with a 7.5 MHz linear-array transrectal transducer, Tokyo, Japan) on d 28.

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

Item	Exp. 1 to 4[1]	Exp. 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, %	6.25	4.50
Calcium diphosphate, %	17.50	—
Calcium carbonate, %	2.50	—
Elemental sulfur, %	0.25	—
Mineral mix, %	5.00[3]	51.5[4]
Sodium chloride, %	20.00	—
Nutrient profile, DM basis		
DM, %	94.1	93.0
TDN, %	38.0	44.0
CP, %	31.6	30.0
NDF, %	4.2	2.9
Ether extract, %	1.2	0.1
Ca, %	5.2	7.7
P, %	3.8	2.0

[1]Values provided by manufacturer (Propec Consultoria Rural Ltda., Campo Grande, Mato Grosso do Sul, Brazil).
[2]Values provided by manufacturer (Lambisk V, Bellman Nutrição Animal Ltda., Mirassol, São Paulo, Brazil).
[3]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.
[4]Values not provided by manufacturer.

Exp. 2

This experiment was designed to compare pregnancy rates of brood cows supplemented with rumen-protected PUFA from the beginning of an estrus synchronization protocol + TAI until before or after expected time of luteolysis (around 16 d after ovulation; Figueiredo

Table 2. Fatty acid (FA) profile of rumen-protected FA sources 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 % of total FA. Values provided by manufacturers.
[2]Megalac-E (Quimica Geral do Nordeste, Rio de Janeiro, Brazil).
[3]Megalac (Church & Dwight Co. Inc., Princeton, NJ).

et al., 1997), and was conducted from December, 2006, to February, 2007, at a commercial cow-calf operation located in Mato Grosso do Sul, Brazil. A total of 818 lactating, primiparous Nelore cows (BCS = 4.7 ± 0.04), approximately 45 to 75 d postpartum, were allocated randomly to 15 *Brachiaria humidicola* pastures (approximately 55 cows/pasture). Pastures were assigned randomly to 1 of the 3 supplement treatments offered from the beginning of synchronization protocol (d −11) until 28 d after TAI (d 28): 1) control supplement offered from d −11 to 28, 2) PF supplement offered from d −11 to 16 and control supplement offered from d 17 to 28 (**PF16**), or 3) PF supplement offered from d −11 to 28 (**PF28**). Treatment feeding and pregnancy status (d 28) verification were conducted similarly as in Exp. 1. Composition and nutritional profile of experimental treatments are described in Tables 1 and 2.

Exp. 3

This experiment was designed to evaluate the effects of rumen-protected PUFA supplementation on serum P_4 concentrations and pregnancy rates to fixed-time embryo transfer of *B. indicus*-crossbred recipient females, and was conducted from November, 2007, to May, 2008, at a commercial embryo transfer center located in Mato Grosso do Sul, Brazil. A total of 435 nulliparous and multiparous recipient cows were stratified by parity and allocated to 14 groups (approximately 31 cows/group). Groups were maintained in *B. decumbens* pastures and were assigned randomly to receive PF or control. Cows were assigned to estrus synchronization + fixed-time embryo transfer protocol, and treatments were offered from the end of synchronization protocol (d 0) until 21 d after embryo transfer (d 28). Because of availability of recipients, groups began receiving treatments at different times during the experimental period (November and December, 2007, 4 groups each month; January to March, 2008, 2 groups each month). Nevertheless, treatment assignment to groups was balanced within each month. Treatment feeding and pregnancy status (d 28) verification were conducted similarly as in Exp. 1. Composition and nutritional profile of experimental treatments are described in Tables 1 and 2. Blood samples were obtained from recipient cows concurrently with embryo transfer (d 7) for determination of serum P_4 concentrations.

Exp. 4

This experiment was designed to compare pregnancy rates and serum P_4 concentrations of brood cows supplemented with rumen-protected sources of PUFA or SFA beginning after TAI. It was conducted from March to May 2008 at 2 commercial cow-calf operations located in Mato Grosso do Sul, Brazil. Across operations, 504 lactating, multiparous Nelore cows (BCS = 4.7 ± 0.01), approximately 40 to 60 d postpartum, were randomly allocated to 12 *B. decumbens* pastures (approximately 42 cows/pasture). Pastures were assigned randomly to receive PF or 0.4 kg/cow daily (as-fed basis) of a protein-mineral mix in addition to 0.1 kg/cow daily of rumen-inert source of SFA (**SF**; Megalac, Church & Dwight Co., Princeton, NJ) for 28 d beginning after TAI (d 0). Treatment feeding and pregnancy status (d 28) verification were conducted similarly as in Exp. 1. Composition and nutritional profile of experimental treatments are described in Tables 1 and 2. Diets were iso-caloric and iso-nitrogenous, but differed in fatty acid profile. Blood samples were obtained from all cows on d 7 after TAI for determination of serum P_4 concentrations.

Exp. 5

This experiment was designed to evaluate the effects of rumen-protected PUFA supplementation on serum concentrations of P_4 and insulin of ovariectomized cows, and was conducted from February to April, 2008, at the São Paulo State University, Lageado Experimental Station, located in Botucatu, São Paulo, Brazil. Nine nonlactating, nonpregnant, and ovariectomized Gir × Holstein cows (BW = 423 ± 17.6 kg; BCS = 4.0 ± 0.06) were stratified by BW and BCS (Wagner et al., 1988) and randomly allocated to 3 squares of 3 cows each. Squares were randomly assigned, in a Latin square 3 × 3 design containing 3 experimental periods of 14 d each, to receive 1 of the 3 supplement treatments (as-fed basis): 1) 0.4 kg of protein-mineral mix + 0.2 kg of kaolin per cow/daily (control), 2) 0.4 kg of protein-mineral mix + 0.1 kg of kaolin + 0.1 kg of a rumen-inert PUFA source (**PF100**; Megalac-E, Quimica Geral do Nordeste) per cow/daily, or 3) 0.4 kg of protein-mineral mix + 0.2 kg of a rumen-inert PUFA source (**PF200**; Megalac-E, Quimica Geral do Nordeste) per cow/daily. Cows were maintained in *Brachiaria brizantha* pastures, whereas treatment feeding was conducted similarly as in Exp. 1. Composition and nutritional profile of experimental treatments are described in Tables 1 and 2. Between experimental periods, cows received no treatments for 7 d to eliminate any residual treatment effects.

Before the beginning of the experiment, cows were inserted with a previously used (3rd use) intravaginal progesterone releasing device (**CIDR**, originally containing 1.9 g of P_4; Pfizer Animal Health, São Paulo, Brazil) from d −7 to 36 h before the beginning of the first experimental period (d 0), to initially expose and adapt cows to exogenous P_4. Cows were inserted with a new CIDR at the beginning of each experimental period (d 0, 22, and 44), which was removed 36 h before the beginning of the following experimental period. Blood samples were collected at the midpoint and also on the last day of each experimental period (d 7 and 14, period 1; d 29 and 36, period 2; d 51 and 58, period 3), immediately before and 2, 4, 6, 8, 10, and 12 h after

treatment feeding, for determination of serum P_4 and insulin concentrations.

Estrus Synchronization and Breeding Procedures

During Exp. 1, 2, and 4, cows received a treatment of estradiol benzoate (2 mg of Estrogin; Farmavet, São Paulo, Brazil) and a CIDR insert on d −11, $PGF_{2\alpha}$ treatment (12.5 mg of Lutalyse; Pfizer Animal Health) on d −4, estradiol cypionate treatment (0.5 mg of **ECP**; Pfizer Animal Health) in addition to CIDR and calf removal on d −2, followed by TAI and calf return on d 0. Within each experiment, all cows were inseminated with semen from the same bull, whereas AI technicians were equally distributed among groups and treatments, to account for potential bull and technician effects.

During Exp. 3, recipient cows received a synchronization protocol similar to that described previously, but instead of calf removal, cows received a 400 IU treatment of eCG (Novormon, Schering-Plough Co., São Paulo, Brazil) on d −2. Transrectal ultrasonography examinations were performed in all recipient cows immediately before embryo transfer (d 7) to verify presence of a corpus luteum. Only cows detected with a visible corpus luteum were sampled for blood and assigned to embryo transfer. All embryos were fresh, originated from in vitro fertilization procedures, and were obtained from a private company (Embyo Sul, Mato Grosso do Sul, Brazil).

During Exp. 1, 2, and 4, BCS was assessed by the same veterinarian on the first day of the estrus synchronization protocol (d −11), according to the procedures described by Wagner et al. (1988).

Blood Analyses

For all experiments, blood was collected via coccygeal vein or artery into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ), placed on ice immediately, maintained at 4°C for 24 h, and centrifuged at 3,000 × *g* for 10 min at room temperature for serum collection; serum was stored at −20°C until further processing. Concentrations of P_4 and insulin were determined using Coat-A-Count solid phase ^{125}I RIA kits (DPC Diagnostic Products Inc., Los Angeles, CA) that were previously validated for bovine samples (Moriel et al., 2008). Samples were analyzed after all experiments were concluded. The intra- and interassay CV were, respectively, 3.7 and 9.7% for P_4, and 5.0 and 7.9% for insulin. The minimum detectable concentrations were 0.1 ng/mL of P_4 and 0.05 μIU/mL of insulin.

Statistical Analyses

Pregnancy rates were analyzed using the GLIMMIX procedure (SAS Inst. Inc., Cary, NC) with a binomial distribution and logit link function. For Exp. 1 and 4, the model statement contained the effects of treatment, ranch, and the interaction. Data were analyzed using pasture(ranch × treatment) as the random variable and error term for the tests of fixed effects. For Exp. 2, the model statement contained the effects of treatment, and data were analyzed using pasture(treatment) as the random variable and error term for the tests of fixed effects. For Exp. 3, the model statement contained the effects of treatment, parity, and the interaction, whereas group(treatment) and month of embryo transfer were the random variables and error terms for the test of treatment effects.

Blood measurements were analyzed with the MIXED procedure of SAS and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. For Exp. 3, the model statement contained the effects of treatment, parity, and the interaction, whereas group(treatment) and month of embryo transfer were the random variables and error terms for the test of treatment effects. For Exp. 4, the model statement contained the effects of treatment, ranch and the interaction, and data were analyzed using pasture(ranch × treatment) as the random variable and error term for the tests of fixed effects. For Exp. 5, the model statement contained the effects of treatment, day, time(day), square, and the appropriate interactions. Data were analyzed using cow(square) and period as random variables. The specified term for the repeated statement was time and the covariance structure utilized was compound symmetry, which provided the best fit for these analyses according to the Akaike information criterion. Further, insulin and P_4 values were tested for normality with the Shapiro-Wilk test from the UNIVARIATE procedure of SAS, and results indicated that all data were distributed normally ($W \geq 0.90$). For comparison of P_4 concentrations in cows with mean insulin above or below the median during the sampling period, data were analyzed with the UNIVARIATE procedure of SAS for median determination and the MIXED procedure of SAS to determine insulin effects on P_4 concentrations. This model statement contained the effects of insulin (above or below median), time, and the interaction. Data were analyzed using cow(period) and period as random variables. The specified term for the repeated statement was time, and the covariance structure utilized was compound symmetry according to the Akaike information criterion.

For all analysis, significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Pregnancy rates are reported as means, whereas blood measures are reported as least squares means. Results were separated using LSD (Exp. 1, 3, and 4), PDIFF (Exp. 5), or single-df orthogonal contrasts (Exp. 2; control vs. PF16 and PF28, PF16 vs. PF28, and PF16 vs. control). Results are reported according to treatment effects if no interactions were significant or according to the highest order interaction detected.

Table 3. Pregnancy rates (pregnant cows/total cows) of beef cows supplemented or not with a rumen-inert PUFA

Item	Pregnancy rate,[1] %	*P*-value
Exp. 1[2]		
Control	39.6 (182/459)	0.04
PF	51.2 (231/451)	
Exp. 2[3]		
Control	31.3 (82/262)[a]	0.05
PF16	35.8 (108/302)[a]	
PF28	42.9 (109/254)[b]	
Exp. 3[4]		
Control	37.7 (78/207)	0.07
PF	49.6 (113/228)	
Exp. 4[5]		
SF	35.5 (85/239)	0.02
PF	47.9 (127/265)	

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

[1]Pregnancy rates are reported as least squares means. Values in parentheses represent number of pregnant cows/total cows.

[2]Cows offered 0.4 kg daily of a protein-mineral mix, in addition to 0.1 kg daily of a rumen-inert PUFA source (PF; Megalac-E, Quimica Geral do Nordeste, Rio de Janeiro, Brazil) or kaolin (rumen-inert indigestible substance; control), from the beginning of synchronization protocol (d −11) to 28 d after timed-AI (d 28).

[3]Control = cows offered 0.4 kg daily of a protein-mineral mix in addition to 0.1 kg daily of kaolin from the beginning of synchronization protocol (d −11) to 28 d after timed-AI (d 28); PF28 = cows offered PF from the beginning of synchronization protocol (d −11) to 28 d after timed-AI (d 28); PF16 = cows offered PF from d −11 to 16 and control from d 17 to 28 relative to timed-AI.

[4]Cows offered PF or control from the end of synchronization protocol (d 0) until d 28 (21 d after embryo transfer).

[5]Cows offered PF or 0.4 kg daily of a protein-mineral mix in addition to 0.1 kg daily of a rumen-inert SFA (SF; Megalac, Church & Dwight Co. Inc., Princeton, NJ) source for 28 d beginning after AI.

Table 4. Serum progesterone concentrations (ng/mL) of beef cows supplemented or not with rumen-inert PUFA

Item	Progesterone	SE	*P*-value
Exp. 3[1]			
Control	3.43	0.396	0.76
PUFA	3.48	0.395	
Exp. 4[2]			
SF	2.91	0.299	0.86
PF	2.84	0.295	
Exp. 5[3]			
Control	1.66[a]	0.142	0.03
PF100	1.81[b]	0.142	
PF200	1.68[a]	0.142	

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

[1]Cows offered 0.4 kg daily of a protein-mineral mix containing in addition to 0.1 kg daily of a rumen-inert PUFA source (PF; Megalac-E, Quimica Geral do Nordeste, Rio de Janeiro, Brazil) or kaolin (rumen-inert indigestible substance; control), from the end of synchronization protocol (d 0) until d 28 (21 d after embryo transfer).

[2]Cows offered PF or 0.4 kg daily of a protein-mineral mix in addition to 0.1 kg daily of a rumen-inert SFA (SF; Megalac, Church & Dwight Co. Inc., Princeton, NJ) source for 28 d beginning after timed-AI.

[3]Control = cows offered 0.4 kg/d of a protein-mineral mix + 0.2 kg/d of kaolin; PF100 = cows offered 0.4 kg/d of protein-mineral mix + 0.1 kg/d of kaolin + 0.1 kg/d of rumen-inert PUFA source (Megalac-E); PF200 = cows offered 0.4 kg/d of a protein-mineral mix + 0.2 kg/d of rumen-inert PUFA source.

For the experiments containing individual BCS data, these values were initially tested as a covariate for pregnancy and P_4 analysis, but no significance or tendencies were detected ($P > 0.10$); therefore, BCS was removed from all models.

RESULTS

Exp. 1

Cows supplemented with PF from the beginning of the estrus synchronization protocol until 28 d after TAI had greater ($P = 0.04$) pregnancy rates compared with control cows (51.2 vs. 39.6% pregnant cows/total cows, respectively; Table 3).

Exp. 2

A treatment effect was detected ($P = 0.05$; Table 3) on pregnancy rates. Cows receiving PF28 had greater ($P = 0.02$) pregnancy rates to TAI compared with control cows (42.9 vs. 31.3% pregnant cows/total cows, respectively) and tended to have greater ($P = 0.10$) pregnancy rates to TAI compared with PF16 cows (35.8% of pregnant cows/total cows in PUFA16). Further, cows receiving PUFA supplementation (39.0% of pregnant cows/total cows in PF16 combined with PF28) had greater ($P = 0.04$; data not shown) pregnancy rates compared with control cows. However, no differences were detected for pregnancy rates between PF16 and control cows ($P = 0.28$).

Exp. 3

No effects of parity or treatment × parity interactions were detected ($P > 0.10$) for pregnancy and P_4 analysis; therefore, the variable parity and the interaction were removed from both models. Recipient cows supplemented with PF for 28 d beginning after the end of the synchronization protocol tended to have greater ($P = 0.07$; Table 3) pregnancy rates to fixed-time embryo transfer compared with control cohorts (49.6 vs. 37.7% pregnant cows/total cows, respectively). No treatment effects were detected for P_4 concentrations ($P = 0.76$; Table 4) on the day of embryo transfer (3.43 vs. 3.48 ng/mL for control and PF cows; SEM = 0.396).

Exp. 4

Cows supplemented with PF for 28 d beginning after TAI had greater ($P = 0.02$) pregnancy rates compared with SF cohorts (47.9 vs. 35.5% pregnant cows/total cows, respectively). No treatment effects were detected ($P = 0.86$; Table 4) for P_4 concentrations (2.91 vs. 2.84 ng/mL of P_4 for SF and PF-supplemented cows, respectively; SEM = 0.30).

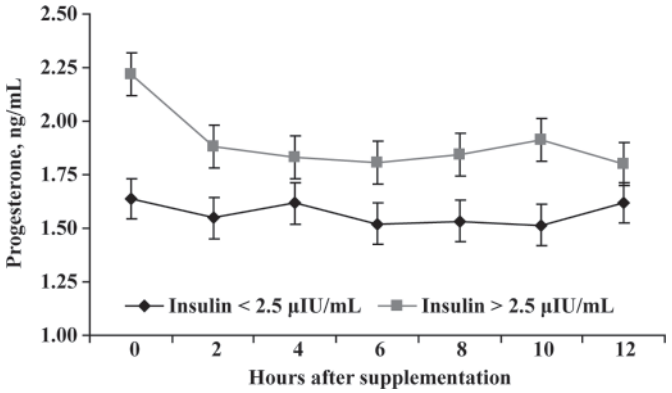

Figure 1. Serum progesterone concentrations (ng/mL) in cows allotted to groups in which mean serum insulin concentrations (μIU/mL) were greater (n = 26) or less (n = 26) than the median concentration (2.5 μIU/mL) during the sampling period. A treatment effect was detected ($P < 0.01$). Cows with mean insulin ≥2.5 μIU/mL had greater mean concentration of progesterone during the sampling period compared with cows with mean insulin concentrations <2.5 μIU/mL (1.89 vs. 1.57 ng/mL, respectively; SEM = 0.098).

Exp. 5

A treatment effect was detected ($P = 0.03$; Table 4) for P_4 analysis. Cows supplemented with PF100 had greater mean P_4 concentrations compared with control ($P = 0.01$) and PF200 ($P = 0.03$) cows (1.81, 1.65, and 1.67 ng/mL, respectively; SEM = 0.14). No treatment effects were detected ($P = 0.46$) for insulin concentrations (2.74, 2.93, and 2.74 μIU/mL for control, PF100, and PF200 cows; SEM = 0.66). When P_4 and insulin were analyzed across treatments, days, and periods, cows having mean insulin concentrations equal or greater than the median (2.5 μIU/mL) during the collection period had greater ($P < 0.01$) mean P_4 concentrations compared with cows with mean insulin <2.5 μIU/mL (1.89 vs. 1.57 ng/mL of P_4; SEM = 0.089; Figure 1).

DISCUSSION

Feeding rumen-protected PUFA to brood cows had beneficial effects on pregnancy rates in all experiments conducted herein, supporting previous reports indicating beneficial effects of PUFA supplementation on reproductive performance of beef cows (Williams and Stanko, 2000; Funston, 2004; Hess et al., 2008). Addition of rumen-protected PUFA to protein-mineral supplements in Exp. 1, 2, and 3 increased pregnancy rates to TAI or to embryo transfer. However, these experiments did not contain a control group offered isocaloric supplements, and the beneficial effects detected for PUFA supplementation cannot be distinguished between the additional energy intake of supplemented cows, or to direct effects of PUFA on cattle reproductive function. The extra energy provided by 0.1 kg of the rumen-inert PUFA source accounted for only 2.8% of the daily TDN requirement of cows utilized in the present experiments (NRC, 1996), which can be considered marginal and insufficient to contribute to the differences detected in pregnancy rates herein. Further, no treatment effects were detected for serum insulin concentrations in Exp. 5, indicating that cows receiving or not 0.1 kg/d of supplemental PUFA likely had similar energy intake, given that circulating concentrations of insulin are typically modulated by rate of energy consumption (Vizcarra et al., 1998).

The results observed in Exp. 4 indicate that PUFA supplementation beginning after TAI increased pregnancy rates in beef cows independent of its contribution to energy intake, given that PUFA cows had greater pregnancy rates to TAI compared with cows supplemented with iso-caloric supplements based on SFA. Results from Exp. 2 also indicate that the beneficial effects of PUFA supplementation on reproductive performance of beef cows can be attributed to its postbreeding effects, more specifically during the period when luteolysis would occur and embryo development would thus be hindered (around d 16 after ovulation in *B. indicus* females; Figueiredo et al., 1997). Mann and Lamming (2001) reported that 85% of serviced cows had a developing embryo within uterine material collected on d 16 after breeding; however, approximately 50% of these cows returned to estrus by d 21 after breeding, indicating that significant embryo losses occur during the expected time of luteolysis. In Exp. 2, pregnancy rates were greater for cows receiving rumen-inert PUFA for 28 d after TAI compared with control cohorts and cows receiving rumen-inert PUFA for only 16 d after TAI, whereas pregnancy rates were similar between control and PF16 cows. Furthermore, recipient cows receiving rumen-inert PUFA for 28 d after the end of the synchronization protocol also had increased pregnancy rates to timed-embryo transfer compared with control cohorts, as observed in Exp. 3.

Beneficial effects of postbreeding PUFA supplementation on pregnancy rates can be attributed to modulation of $PGF_{2\alpha}$ synthesis and luteolysis (Williams, 1989; Williams and Stanko, 2000; Funston, 2004), enhanced maternal recognition of pregnancy (Wathes et al., 2007), and also increased circulating P_4 concentrations. Concentrations of P_4 after breeding are positively associated with pregnancy rates in beef and dairy cattle (Robinson et al., 1989; Stronge et al., 2005; Demetrio et al., 2007). This relationship can be explained by the fact that P_4 prepares the uterine environment for conceptus growth and development, modulates the release of hormones that may regress the corpus luteum and disrupt gestation (Bazer et al., 1998), and thus is required for proper establishment of pregnancy (Spencer and Bazer, 2002). Moreover, P_4 regulates endometrial secretions that are essential for posthatching development (Gray et al., 2001) and also endometrial structural changes that are essential for proper embryo development (Wang et al., 2007). Postbreeding PUFA supplementation can increase circulating P_4 concentrations by increasing circulating cholesterol concentration, which is the major precursor for luteal P_4 synthesis (Grummer and Carroll, 1991; Son et al., 1996), but also by directly allevi-

ating hepatic steroid metabolism (Hawkins et al., 1995; Sangsritavong et al., 2002). This latter mechanism is supported by the results observed in Exp. 5, indicating that supplementation of rumen-inert PUFA, at 0.1 kg/cow daily, likely reduced hepatic clearance of serum P_4, given that cows were nonlactating and ovariectomized. The reasons for similar effects not occurring when cows were supplemented with 0.2 kg/d of the same rumen-inert PUFA are unknown and warrant further investigation. No treatment effects were detected for P_4 concentrations in Exp. 3 and 4, when blood samples were collected on d 7 after TAI. However, cows from Exp. 3 and 4 were sampled for blood once throughout the day without any specific order in relation to treatment feeding time, whereas cows from Exp. 5 were sampled serially after treatment feeding. Circulating concentrations of P_4 are highly affected by feed intake (Vasconcelos et al., 2003; Cooke et al., 2007), which may explain why similar treatment effects on serum P_4 concentrations were not observed across all experiments. Supporting the results from Exp. 5, Hawkins et al. (1995) reported that beef cows supplemented with rumen-protected fat sources experienced reduced rate of serum P_4 disappearance after ovariectomy compared with cows supplemented with iso-caloric and iso-nitrogenous treatments containing no supplemental fat. Sangsritavong et al. (2002) reported that incubation of liver slices with P_4 in the presence of linoleic acid increased P_4 half-life in the media culture. Sangsritavong et al. (2002) also reported that hepatic clearance of P_4 was reduced in nonlactating dairy cows infused with an emulsion of soybean oil, and concluded that specific fatty acid supplementation can increase circulating P_4 concentrations by directly inhibiting liver steroid metabolism.

Supplementation with PUFA may also influence uterine synthesis of $PGF_{2\alpha}$ (Lucy et al., 1991; Mattos et al., 2002) and consequently affect corpus luteum lifespan, circulating P_4 concentrations, and maintenance of the pregnancy. Elevated concentrations of $PGF_{2\alpha}$ after breeding promote luteolysis and also have direct embryotoxic effects (Inskeep, 2004). Linoleic acid can be desaturated and elongated to arachadonic acid, and thus serve as a precursor for $PGF_{2\alpha}$ synthesis. However, previous reports indicate that linoleic acid can hinder $PGF_{2\alpha}$ synthesis by competitive inhibition of the enzymes Δ^6-desaturase and cyclooxygenase (Staples et al., 1998; Cheng et al., 2001). Still, others have reported that supplementation with linoleic acid sources after breeding increased circulating concentrations of $PGF_{2\alpha}$ metabolite and reduced pregnancy rates in beef cows (Filley et al., 2000; Grant et al., 2005). Supplementation with linolenic acid, on the other hand, inhibits synthesis of $PGF_{2\alpha}$ and reduces pregnancy losses in cattle (Pace-Asciak and Wolfe, 1968; Thatcher et al., 1994; Ambrose et al., 2006). Linolenic acid is a precursor of eicosapentaenoic and docosahexaenoic acids, which have been shown to inhibit cyclooxygenase activity and consequently $PGF_{2\alpha}$ synthesis in the endometrium, delaying luteolysis, and thus improving pregnancy rates in dairy cows (Burke et al., 1997; Thatcher et al., 1997; Mattos et al., 2000). The rumen-inert PUFA source offered to cattle in the present study contained linoleic and linolenic acids in significant amounts. According to the CPM-Dairy model (Cornell-Penn-Miner Dairy, version 3.08.01; University of Pennsylvania, Kennett Square; Cornell University, Ithaca, NY; and William H. Miner Agricultural Research Institute, Chazy, NY), the PF treatment provided 12.91 g/d of linoleic and 0.83 g/d of linolenic acid that were absorbable by the duodenum of treated cows, whereas the SF treatment provided, respectively, 2.46 and 0.07 g/d of absorbable linoleic and linolenic acid, and the control treatment provided, respectively, 0.24 and 0.01 g/d of absorbable linoleic and linolenic acid. Thatcher and Staples (2000) suggested that the amount and type of fatty acids reaching the target tissues likely influence whether $PGF_{2\alpha}$ synthesis is stimulated or inhibited. Thus, it cannot be concluded if cows offered supplemental rumen-inert PUFA experienced greater reproductive performance compared with control or SFA-supplemented cows because of additional supply of linoleic acid, linolenic acid, or both. In addition, it cannot be determined if treatment effects on pregnancy rates were mediated directly by PUFA or via its effects on circulating P_4 concentrations, as detected in Exp. 5.

Previous studies indicated greater circulating insulin concentrations in cattle receiving supplemental PUFA compared with nonsupplemented cohorts (Williams and Stanko, 2000). Elevated insulin concentrations reduce hepatic expression of P_4 catabolic enzymes P450 2C and P450 3A (Murray, 1991; Lemley et al., 2008), which can further increase circulating P_4 concentrations. However, no treatment effects were detected in Exp. 5 for insulin concentrations, indicating that PUFA supplementation increased serum P_4 concentrations independently of insulin-associated mechanisms. Nevertheless, cows with elevated insulin concentrations during the sampling period concurrently had greater mean P_4 concentrations compared with cows with reduced insulin concentrations, supporting previous data from our research group indicating that peripheral concentrations of insulin potentially modulate hepatic clearance of P_4 (Moriel et al., 2008).

In conclusion, interactions between the corpus luteum, endometrium, and embryo are critical to the successful establishment of pregnancy. Results from these studies indicate that supplementation of rumen-inert PUFA sources to beef females is a strategy to increase pregnancy rates, and these outcomes can be attributed, at least in part, to beneficial postbreeding effects of PUFA on reproductive function. Furthermore, ovariectomized cows supplemented with exogenous P_4 and offered 100 g of a rumen-inert PUFA source experienced greater serum P_4 concentrations compared with cohorts not offered supplemental fat, and this outcome is likely due to reduced hepatic P_4 metabolism in PUFA-supplemented cows. Therefore, PUFA supplementation may increase reproductive performance of beef cows

by directly improving uterine environment and embryo development, perhaps by increasing circulating concentrations of P_4.

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