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Supplementation with sunflower seed increases circulating cholesterol concentrations and potentially impacts on the pregnancy rates in *Bos indicus* beef cattle

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ABSTRACT

We aimed to evaluate the effect of supplementation with sunflower seed on blood concentrations of progesterone and cholesterol and on the pregnancy rate in beef cattle subjected to timed artificial insemination (TAI) and timed embryo transfer (TET). In experiment 1, cows were received 22-day supplements containing (sunflower, n = 66) sunflower seed or not (control, n = 67) immediately after a progesterone/estradiol–based TAI protocol (Day 0). The cholesterol concentration on Day 21 and the pregnancy rate were greater ($P < 0.03$) in the sunflower group (148.2 ± 6.1 mg/dL and 66.7%) than those in the control group (116.0 ± 6.4 mg/dL and 47.8%). In experiment 2, heifers received an *in vitro*–produced embryo 7 days after the expected time of the synchronized ovulation. Heifers were separated into two supplementation groups (sunflower, n = 106 and control, n = 111) for 22 days. The plasma progesterone concentration on Day 7 was not different between the groups. However, on Day 19, the plasma progesterone concentration was greater ($P < 0.0001$) in the sunflower group (5.8 ± 0.4 ng/mL) than that in the control group (3.5 ± 0.4 ng/mL). A greater ($P < 0.05$) cholesterol concentration was observed in the sunflower group than that in the control group on Days 7 (306.0 ± 11.6 vs. 277.1 ± 11.9 mg/dL, respectively) and 19 (260.5 ± 8.0 vs. 232.0 ± 8.0 mg/dL, respectively). The pregnancy rate was greater ($P = 0.01$) in the sunflower-treated heifers (55.7%) than that in control-treated heifers (36.9%). Results indicate that sunflower seed supplementation increases the circulating cholesterol concentrations and potentially impacts the pregnancy rate in suckled beef cattle subjected to TAI or TET.

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1. Introduction

Early embryo mortality is an important cause of reproductive failures in cattle. Specifically, 20% to 40% of embryonic losses occur between the 15th and 19th days of pregnancy in cattle [1,2]. The suppression of endometrial PGF2α secretion is required for the establishment of pregnancy during this period [3].

Previous studies reported beneficial effects of supplementation with linoleic acid, a source of fatty acid, on the synthesis and secretion of PGF2α [4], development of ovulatory follicle and CL, embryo development, and interferon-τ production [5–9]. Furthermore, fatty acid supplementation has been associated with greater concentrations of cholesterol in circulatory system, follicular fluid, and corpora lutea [5,10–13], which indicate a greater capacity for 17-β estradiol and progesterone (P4) synthesis. In this regard, increased circulating P4 concentrations within the first weeks after conception are associated with altered gene expression in the endometrium [14], which results in advancement in conceptus elongation [15,16]. In addition, circulating P4 concentrations are positively associated with the conception likelihood in dairy and beef cattle [17,18].

Improvements on the conception rate using supplementation with polyunsaturated fatty acid were also reported in beef and dairy cows [19,20]. However, the use of sunflower seeds (*Helianthus annuus*), a rich source of polyunsaturated fatty acid, as a basis of fat supplementation has been poorly studied in cattle. A satisfactory pregnancy rate (≈50%) has been achieved in commercial timed artificial insemination (TAI) programs in Brazil [21,22]. Despite to this reasonable result, the ovulatory rate achieved in those females after the synchronization of estrus and ovulation protocols is comparatively greater (85%–90%) than the conception rate [23]. This discrepancy indicates that a possible failure of fertilization or occurrence of early pregnancy loss in those inseminated females that failed to become pregnant [1,2,24]. In this same context, the conception rate even after timed embryo transfer (TET) generally does not exceed 50% [25–27], reinforcing the possible role of embryonic loss as a possible limiting factor to improvements in estrous synchronization programs. Thus, the development of strategies to reduce early embryo mortality in cattle, such as the use of nutraceutical products, could be considered important to improve the reproductive efficiency and the livestock production.

Therefore, we aimed in this study to evaluate the effects of sunflower seed supplementation on blood concentrations of P4 and cholesterol and the consequence on the 30-day pregnancy rate of beef cattle submitted to TAI (experiment 1) or TET (experiment 2). We hypothesized that sunflower seed supplementation increases the blood concentrations of P4 and cholesterol, which may improve the 30-day pregnancy rate in cattle submitted to TAI or TET. Furthermore, we also aimed to establish the effects of sunflower supplementation for a short period (3 weeks) on circulating concentrations of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) during the maternal recognition of pregnancy period.

2. Materials and methods

2.1. Experiment 1: TAI in suckled beef cows

2.1.1. Farm and cows

Experiment 1 was conducted in a commercial beef farm (Fazenda Guanabara, Grupo Agropecuária J. Galera), located in the state of Goiás, Brazil, using suckled beef cows. Nelore cows (n = 133) between 40 and 130 days postpartum, weighing between 410 and 510 kg (average, 471 ± 7.8 kg) and with a body condition score (BCS) of 3.74 ± 0.32 (scale 1–9), were maintained on pastures (*Brachiaria brizantha*) with water and mineral salt *ad libitum*. The calves were kept with their dams and were fed a supplement containing soybean meal and corn in a creep feeding system. The females were blocked by days postpartum period, body weight at TAI, BCS at TAI (1–9), gender of the calf, and ovarian status and were randomly assigned into one of two groups (sunflower or control). The days postpartum period was calculated on the basis of the period between the day of the partum and the beginning of the protocol for estrous synchronization.

The ovarian structures of the females were also assessed 24 hours before the beginning of the TAI protocol and classified as follows: presence of a CL (13.5%; n = 18), absence of CL and presence of large follicles (≥10 mm; 71.4%; n = 95), and absence of CL and presence of small follicles (<10 mm; 15.0%; n = 20). All experimental procedures were approved by the Committee of Ethics and Animal Experimentation from the São Paulo State University (UNESP), Dracena campus, Dracena/SP, Brazil (Protocol number 04/2009).

2.1.2. Synchronization of estrus and ovulation for TAI

The cows received an intravaginal P4 device containing 1.0 g of P4 (Cronipress; Biogenesis Bago, Curitiba, PR, Brazil) along with 2 mg of estradiol benzoate intramuscular (2-mL Bioestrogen; Biogenesis Bago) on the first day of the synchronization protocol (Day −10; Fig. 1). The devices were removed 8 days later (Day −2), at which time the cows received 150 μg of d-cloprostenol intramuscular (2 mL; Croniben; Biogenesis Bago) and 400 UI of eCG (2 mL; Folligon; Intervet, Summit, NJ, USA). Ovulation was induced by a second estradiol benzoate treatment (1 mg) administrated 24 hours after the removal of the P4 device (Day −1), and TAI (Day 0) was performed by a single technician 30 hours after the last estradiol injection. Semen from 16 proven fertility bulls was equally distributed within each experimental group.

2.1.3. Sunflower seed supplementation

Immediately after the TAI, each experimental group was put in neighboring paddocks with similar forage quality and availability. At this same day, cows were randomly assigned into one of the two groups to receive 1.7 kg/animal/day of one of the following treatments: 40% soybean meal containing 44% crude protein (CP) plus 60% of sunflower seed (sunflower group, n = 66) or 53% soybean meal containing 44% CP plus 47% corn (control group, n = 67). Both supplements were balanced with 72% total digestible nutrients and 24% CP and were given for 22 days after the