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Original Research Paper

Dietary Inclusion of Sunflower Seed Alters Blood Plasma and Endometrium Lipid Profile in Beef Cows

[1]Mariângela Bueno Cordeiro Maldonado, [2]Ricardo de Oliveira Rodrigues, [3]Dante Pazzanese Duarte Lanna, [4]Guilherme Pugliesi, [5]Milton Maturana Filho, [6]Adriano Felipe Mendes, [6]Lucas de Oliveira Bezerra, [7]Ricardo da Fonseca and [7]Claudia Maria Bertan Membrive

[1]Department of Biological Sciences, São Paulo State University (UNESP-FCL), Brazil
[2]Provimi, Cargill Animal Nutrition, Lewisburg, Pensilvânia, USA
[3]Department of Animal Science, University of São Paulo (USP-ESALQ), Piracicaba, São Paulo, Brazil
[4]Department of Animal Reproduction, University of São Paulo (USP-FMVZ), Pirassununga, São Paulo, Brazil
[5]MF Vetplan, Águas da Prata, São Paulo, Brazil
[6]Department of Biophysics and Pharmacology, São Paulo State University (UNESP-IBB), Botucatu, São Paulo, Brazil
[7]Department of Animal Production, São Paulo State University (UNESP-FCAT), Dracena, São Paulo, Brazil

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Corresponding Author:
Mariângela Bueno Cordeiro Maldonado
Department of Biological Sciences, São Paulo State University (UNESP-FCL), Brazil
Email: mariangela.maldonado@unesp.br

Abstract: Polyunsaturated Fatty Acids (PUFA)-enriched diets are recognized as a significant approach for improving the reproductive efficiency of cattle. The aim of this study was to evaluate the effects of sunflower seed, a PUFA-rich feed source, supplementation on plasma and endometrium lipid profiles in beef cattle. It was hypothesized that feeding of sunflower seed has an additional effect on PUFA that causes changes in the profiles of total cholesterol, Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), and triglycerides plasma concentrations, and endometrium fatty acids composition. For such, 60 Nelore cows had their ovulation synchronized and were then fed 1.7 kg/day/animal of control diet composed of soybean meal and corn or enriched with sunflower seed. Both diets were provided in troughs maintaining the measure of 22 linear cm/trough/animal. The cows were supplemented for 6 (D0-D5), 14 (D0-D13), or 22 days (D0-D21), according to the experimental group, from the expected estrus (D0) and blood was collected throughout the treatment. 24 h after receiving the last supplementation, 15 control, and 15 treated cows were slaughtered for analysis of blood plasma and endometrium lipid profile. The plasma lipid concentrations were assessed through the utilization of commercially available colorimetric kits employings an enzymatic method in an automated analyzer. The endometrial fatty acids profile was analyzed by gas chromatography. Treated cows presented increased total plasma cholesterol concentrations on D18 and D21; Increased HDL concentrations on D10, D14, D18, and D21; and increased LDL concentrations on D21 ($p<0.05$); but no difference in triglycerides. Furthermore, feeding sunflower seed to beef cows increased endometrial concentrations of C18:1 T10-T11-T12 and C10:1 and decreased those of iso-C15:0, C20:5, C20:3 n3, C23:0, C24:0 and C22:5 fatty acids. We conclude that feeding beef cows with 1.7 kg/day/animal of sunflower seed alters the lipid profile of plasma and endometrium and that such changes are potentially associated with higher reproductive efficiency.

Keywords: Linoleic Acid, Lipid, Prostaglandin, Embryonic Mortality, Cattle

Introduction

Enhanced conception rates in beef and dairy cows were observed with the addition of Polyunsaturated Fatty Acids (PUFAs) as a supplement. Santos *et al*. (2008); Lopes *et al*. (2009); Elis *et al*. (2016). Nevertheless, there has been limited research on the utilization of sunflower seed (*Helianthus annuus*) as a source of fat supplementation in cattle, despite their high content of PUFAs.

PUFA-enriched diets promote benefits in follicular development (Staples and Thatcher, 2006), and improve fertilization rate and embryo quality, and developmental progress (Thangavelu *et al*., 2007; Santos *et al*., 2008).

Science Publications

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They can affect the functional and physical characteristics of membranes, as well as affect molecular signaling and gene expression. (Calder, 2012). Interestingly, our previous study (Cordeiro *et al*., 2015) reported that 22 days of sunflower seed supplementation to beef cows subjected to Timed Artificial Insemination (TAI) or Timed Embryo Transfer (TET) increased conception rate by about 19% when verified that such increase was not associated with higher circulating concentrations of progesterone.

Associated effects could be observed in beef cows, which experienced increased conception rate when cows were supplemented with PUFA-rich diet ingredients such as protected fats during postpartum (Ferguson *et al*., 1990; Garcia-Bojalil *et al*., 1998; McNamara *et al*., 2003) after and before TAI and TET (Lopes *et al*., 2009; Cordeiro *et al*., 2015) and from second to fourth TAI (Sklan *et al*., 1991). In dairy cattle, the addition of PUFAs to the diet is implemented to increase the energy concentration. Typically, the sources of these fatty acids are supplied in the form of oil or fat, given the considerably higher energy concentration of PUFAs compared to other dietary components, up to 2.5 times higher (Carneiro *et al*., 2021). Furthermore, specific fat sources offer benefits beyond their high energy content, as they exert positive effects on various physiological processes (Rosero *et al*., 2016).

Biological challenges for pregnancy maintenance occur mostly between 15 and 17 days after fertilization (Binelli *et al*., 2001) when 20-40% of embryonic losses are reported in cows (Vanroose *et al*., 2000). During this critical period, the conceptus-embryo and its appendages (adnexa)-secretes molecules that interact with the endometrium, suppressing prostaglandin-F2α (PGF2α) synthesis, consequently preventing luteolysis. However, only the conceptus' presence in the uterine lumen does not ensure luteolysis prevention. The inclusion of n-3 PUFAs in the diet inhibits the synthesis of bovine endometrial PGF2α, both *in vitro* and *in vivo* (Mattos *et al*., 2002), which consequently leads to improved fertility and increased embryonic survival (Soydan *et al*., 2017). The mechanism through which n-3 PUFAs inhibit PGF2α remains unknown. It is possible that PUFA supplementation alters the lipid profile of the endometrial tissue by restricting the amount of arachidonic acid, the main precursor of PGF2α, thus limiting the synthesis of PGF2α of the endometrium during maternal recognition of pregnancy thus improving reproductive rates (Mattos *et al*., 2000; Petit *et al*., 2004; Lopes *et al*., 2009).

Thus, we hypothesized that supplementation with a PUFA-rich ingredient post-estrus alters plasma and endometrium lipid profiles in beef cows. Our objective was to evaluate the effects of supplementing with sunflower seed, a PUFA-rich feed source, on plasma and endometrium lipid profiles.

Materials and Methods

Animals

All experimental procedures were reviewed and approved by the Ethical Committee for Animal Use in Research from São Paulo State University (UNESP), Faculty of Agricultural and Technological Sciences, Dracena/SP, Brazil (protocol number 14/2013). The experiment was conducted at Santa Encarnação Farm, a commercial cow-calf beef operation located in Santa Rita do Pardo, MS, Brazil, using seventy multiparous, non-lactating Nelore cows, averaging and Standard Error of the Mean (SEM) 397.5±6.4 kg of Body Weight (BW) and averaging and SEM 3.4±0.1 of Body Condition Score (BCS) (scale 1 to 5). On the day of initiation of the protocol, for synchronization of ovulation (D-10 from expected estrus) ovarian structures were evaluated by ultrasonography where the diameter of the largest follicle and the presence or absence of corpus luteum were considered (Aloka Ultrasound Diagnostic Equipment, Model SSD-500; Tokyo, Japan) and classified as Presence of a CL (n = 32), absence of CL and presence of large follicles (≥10 mm; n = 33) and absence of CL and presence of small follicles (<10 mm; n = 5). Cows were ranked by BW, BCS, and ovarian status and were divided equally into two groups to receive a dietary supplement enriched with sunflower seed (treated group; n = 35) or not (control group; n = 35). Then, cows within a dietary treatment were distributed into three distinct groups to evaluate the timing effects of dietary supplementation on altering the lipid profile of plasma and endometrium. Cows were maintained on pastures (*Brachiaria decumbens* cv. *Basilisk*) with mineral salt and water ad libitum throughout the trial.

Ovulation Synchronization and Monitoring

Cows (n = 70) were synchronized using a progesterone device containing 1.0 g of P4 (DIB®, Syntex Biochemistry, and Pharmaceutical) placed intravaginally along with 2 mg of estradiol benzoate injected Intramuscularly (IM) (Benzoato HC®, Benzoate HC, Hertape Calier animal health) on the first day of the synchronization protocol (d-10 from expected estrus; Fig. 1). The progesterone devices were taken out after 8 days (day-2) when cows received 2 mg of sodium cloprostenol IM (Sincrocio®, Ourofino animal health, Cravinhos, SP, Brazil), 0,5 mg of estradiol cypionate (ECP®, Ourofino animal health, Cravinhos, SP, Brazil) and 300 UI of equine chorionic gonadotropin (eCG; Folligon; Intervet, Summit, NJ, USA). After progesterone device removal, the tail-head of each cow was painted using a paint stick (Walmur,

Porto Alegre, RS, Brazil) to assist in estrus detection. Cows were monitored twice daily for estrus signs from 36-96 h after the removal of the P4-releasing device. Daily records were maintained for paint scores on a scale of 0-5 (no change in color = 5; complete disappearance of color = 0). Cows observed in standing estrus and/or no signs of paint in their tailheads were considered in estrus (D0 = estrous day). Between D1 and D3 (D0 = estrous day), the ovaries were evaluated daily by ultrasonography scanning (Aloka ultrasound diagnostic equipment, model SSD-500; Tokyo, Japan) to determine ovulation through the disappearance of the dominant follicle. The ovulation rate was 85% after the synchronization of ovulation (n = 60).

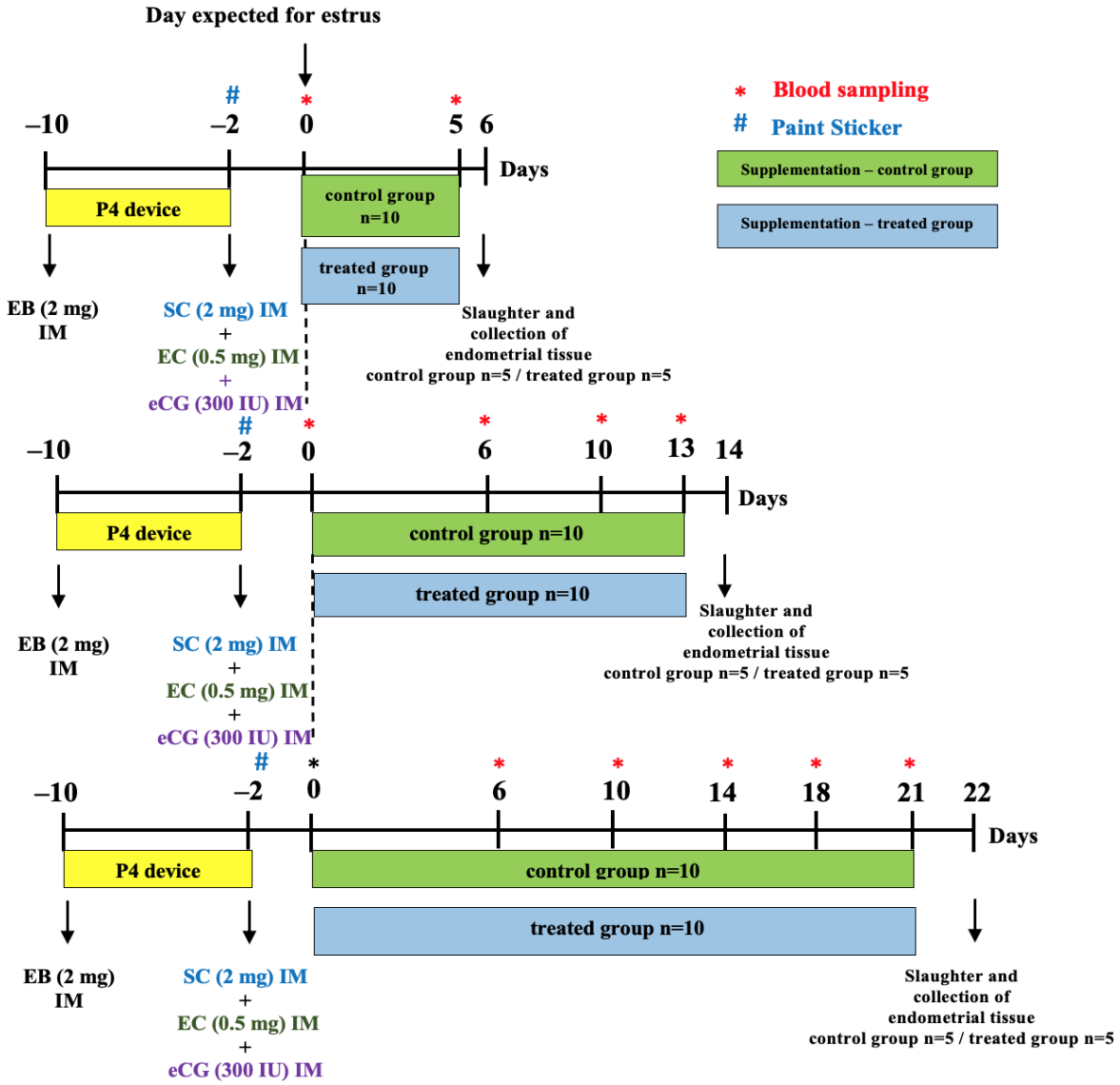

Fig. 1: Schematic representation of the experimental design. Cows received an intravaginal progesterone-releasing device and estradiol benzoate on the first day of the synchronization protocol (D -10). Eight days after removal of the device (D -2), cows received Sodium Cloprostenol (SC), Estradiol Cypionate (EC), and Equine Chorionic Gonadotrophin (eCG) all injected Intramuscularly (IM). The diets, enriched with sunflower seed (treated group) or standard non-supplemented (control group), started on the day expected for estrus (D0). Animals were treated for 6 (6-D; D0 to D5), 14 (14-D; D0 to D13), or 22 days (22-D; D0 to D21). We collected blood sampling on D0 and D5 (6-D); or D0, D6, D10 and D13 (14-D); or D0, D6, D10, D14, D18 and D21 (22-D). Females were slaughtered 24 h after supplementation periods with the animals fasting. Endometrial tissue was collected after the slaughter

Experimental Design

Timing groups consisted of cows supplemented accordingly for 6, 14, or 22 consecutive days, beginning on an estrous day (D0) following synchronization of ovulation from D0 to D5, D0 to D13, or D0 to D21(Fig. 1). The cows were not inseminated and only cows that had ovulated following synchronization of ovulation (85%, n = 60) remained in the experiment. Thus, 10 cows per dietary treatment per timing group were ultimately evaluated. This research also aimed in unveiling changes in the fatty acids profile of the endometrium. For such, a subset of 5 cows per dietary treatment per timing group was randomly selected and slaughtered fasting 24 h after the last day of dietary supplementation, on D6, D14, and D22 (D0 = estrous day). Because cows were slaughtered in a commercial facility, all procedures, including the beginning of the trial for each specific timing group, were planned so that cows would be slaughtered on the same date. On the day of treatment assignment, each experimental group was assigned to adjacent paddocks that provided comparable forage quality and availability. Once daily, treated cows received 1.7 kg/cow (as-fed basis) of a supplement consisting of 40% soybean meal 44% Crude Protein (CP) plus 60% of sunflower seed; control cows received similar amounts of an isoenergetic (72% total digestible nutrients) and isoproteic (24% CP) supplement consisting of 53% soybean meal 44% CP plus 47% corn. Both diets were provided in troughs maintaining the measure of 22 linear cm/trough/animal. To mitigate the potential impact of slight changes in pasture composition between the paddocks, cows within treatment groups were rotated between the two paddocks every 5 days.

Blood Collection and Plasma Lipid Concentrations

Blood samples were collected by venipuncture of the coccygeal vessels on A) D0 (before initiation of dietary treatment) and D5 from cows supplemented for 6 days, B) D0, D10, and D13 from cows supplemented for 14 days, and C) D0, D6, D10, D14, D21 from cows supplemented for 22 days. Blood samples were collected in a 4 mL acrylic vacuum tube (Vacuntainer systems, Becton, Dickinson, Franklin Lakes, NJ, USA) containing ethylenediamine tetraacetic acid, and samples were stored at 4°C until centrifugation. Samples were centrifuged at 2,900×*g* for 30 min at room temperature. Plasma was then separated and stored at-20°C until further analysis. An enzymatic method was applied to measure plasma lipid composition using an automated analyzer (RX Daytona®, Randox Laboratories, UK), as previously reported by our laboratory (Cordeiro *et al*., 2015). Briefly, total plasma cholesterol concentrations were determined using the CHOL R1 kit (Randox Laboratories Ltda, REF CH 3810, Lot 557CHRX). Reference samples Containing High (HC), Intermediate (IC), and Low Concentrations (LC) of cholesterol were used. All samples were measured in a single assay. The intra-assay coefficients of variation were 3.73, 1.67, and 3.84% for HC, IC, and LC, respectively. The plasma triglyceride concentrations were determined using the TRIgs R1b Kit (Randox Laboratories Ltda, REF TR3823, Lot 83TRRX) according to (Fossati and Prencipe, 1982). The plasma HDL concentrations were determined using the HDL R2 kit (Randox Laboratories Ltda, REF CH 3811, Lot 609CHRX). Reference samples HC, IC, and LC of HDL were used. All samples were measured in a single assay. Intra-assay coefficients of variation were 1.80, 1.45, and 3.11% for HC, IC, and LC, respectively. Concentrations of LDL in plasma were calculated using the Friedewald equation (Friedewald *et al*., 1972):

$$LDL\frac{mg}{dL} = \left[totalcholesterol - HDL - \left(\frac{triglycerides}{5} \right) \right]$$

Endometrial Tissue Collection and Fatty Acids Profile Analysis

Genital tracts were collected and uterine horns ipsilateral to the corpus luteum were sectioned longitudinally to expose the endometrial tissue within 1 h after slaughtering. The endometrium was then dissected and three repetitions were stored in cryovials at-96°C until further analysis. To ascertain the profile of fatty acids in the endometrium, the total lipids were extracted using a combination of organic solvents (hexane: Isopropanol; 3:2 v/v) as described by Hara and Radim (1978). The lipid fraction was methylated with a basic solution of methoxide sodium, according to Christie (1982). Trans-methylated samples were analyzed in a gas chromatograph model focus CG-Finnigan, with flame ionization detector and CP-Sil 88 (Varian) capillary column, 100 m long by 0.25 μm internal diameter and 0.20 μm of film thickness. Hydrogen was used as a carrier gas at a flow rate of 1.8 mL/min. Samples were submitted to increasing temperatures up to 215°C for a total of 65 min; the temperature of the vaporizer was set to 250°C and the detector was set at 300°C. The esterified extract was injected into the chromatograph and fatty acids were identified through a comparison of the retention times of the methyl esters in the samples with fatty acid standards. The normalized area under the curve for methyl esters was used for quantification. Chromquest 4.1 software (Thermo electron, Italy) was used for the relative quantification of fatty acids. The endometrium samples

collected from individual cows contained insufficient amounts of lipids for proper and accurate profiling of fatty acids. As a consequence, our laboratory decided on pooling all samples pertaining to cows within each dietary treatment (5 cows per timing group, totaling 15 cows per dietary treatment) based on equal contributions, thus creating two pools, one for the treated group and one for the control group with 3 repetitions per group.

Statistical Analysis

Statistical analysis only included data from cows that remained in the experiment. Analysis was performed using a statistical analysis system (version 9.1, SAS Institute Inc., Cary, NC, USA). Continuous variables were tested for normality using the guided data analysis tool from SAS and, when necessary, transformed according to the software's suggestion to achieve normality. Further, data were analyzed based on two-way ANOVA, using the procedure GLIMMIX. A single model considering the effects of treatment, time, and their interaction was used for data analysis. For evaluation of repeated measurements of plasmatic components used only the model considers the effects of treatment, time, and their interaction. Post-hoc tests, when needed, were realized by t-test. Values are shown as mean ± SEM. Statistical significance was considered when $p \leq 0.05$ and the tendency was considered at $0.05 < p \leq 0.1$.

Results

Circulating Lipid Profile

For cholesterol, concentrations were observed an interaction between treatment and time reflected higher total cholesterol plasma concentrations in the treated group on D18 (150±7.9 vs. 13±7.3 mg/dL) and D21 (161.6±6.7 vs. 142.3±8.2 mg/dL) than in the control group ($p<0.05$; Fig. 2A).

For plasma HDL concentrations, an interaction between treatment and time was observed, which reflected in higher HDL concentrations in the treated group on D10 (41±1.7 vs. 37.1±1.6 mg/dL), D14 (41.8±1.8 vs. 36.9±1.9 mg/dL), D18 (43.3±1.9 vs. 38.8±2.1 mg/dL) and D21 (45.2±1.5 vs. 39.9 ± 2.3 mg/dL) than in control ($p<0.05$; Fig. 2B).

In our study, no group effect or interaction between treatment and time was observed for the plasma triglyceride concentrations.

Moreover, we observed an interaction between treatment and time for plasma LDL concentrations, which resulted in higher plasma LDL concentration in the treated group on D21 than in the control (111.2±5.4 vs. 96.8±6.3 mg/dL; $p<0.05$; Fig. 2C).

Points where there was an interaction ($p<0.05$) between treatment and supplementation day.

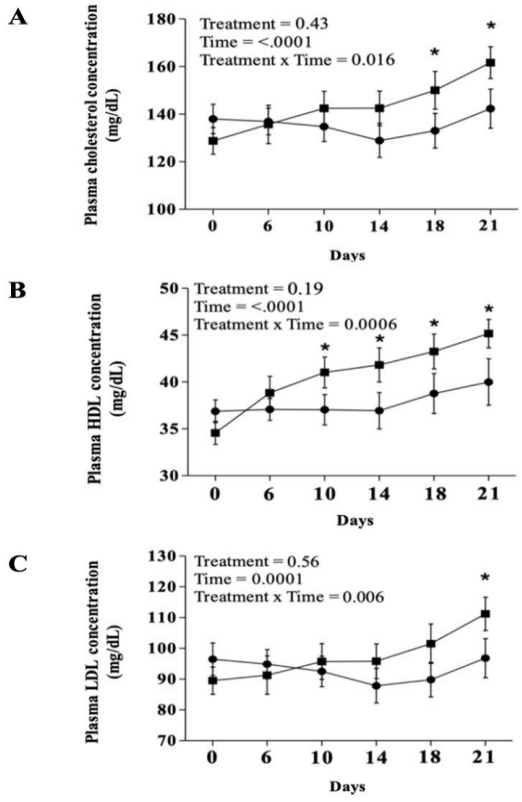

Fig. 2: Effect of treatment, time and interaction between treatment × time on plasma concentrations of cholesterol (A), HDL (B), and LDL (C) on D0, D6, D10, D14, D18, and D21 of Nelore cows fed 1.7 kg/animal/day of the diet supplemented with sunflower seed (sunflower group; n = 15) or standard diet (control group; n = 15) for 6 (6-D; D0 to D5), 14 (14-D; D0 to D13), or 22 days (22-D; D0 to D21) starting immediately after removal of the device (D0)

Endometrium Fatty Acids Profile

Out of the 54 fatty acids evaluated by gas chromatography, we observed 43 in the endometrial tissue of females (Table 1). Our analysis detected that eight of them showed differences in relative abundance between the treated group and the control group (Table 1). The fatty acids that showed higher relative abundances in the treated group than in the control were trans-10,11,12-Octadecenoic acid (C18:1 T10-T11-T12; $p = 0.014$) and Decenoic acid C10:1 ($p = 0.086$). In contrast, we observed a lower abundance of Isopentadecanoic acid (iso-C15:0; $p = 0.05$), Eicosapentaenoic (EPA; C20:5; $p = 0.04$), n3-Eicosatrienoic (C20:3 n3; $p = 0.05$), Tricosanoic acid (C23:0; $p = 0.08$), Tetracosananoic acid (C24:0; $p = 0.08$) and Docosapentaenoic (C22:5; $p = 0.08$) in the treated group than in the control. The fatty acids that were not present in none of the groups in the referred tissue were Butanoic acid (C4:0),

Undecanoic acid (C11:0), Lauroleic acid (C12:1), Tridecanoic acid (C13:0), Isotridecanoic acid (iso-C13:0), Anteisotridecanoic acid (anteiso-C13:0), Isomyristic acid (iso-C14:0), Pentadecenoic acid (C15:1), trans-16-Octadecenoic acid (C18:1 T16), cis-12-trans-10-Octadienoic acid (C18:2 T10 C12) and Heneicosanoic acid (C21:0).

Table 1: Mean (± SEM) of 43 short, medium, and long-chain fatty acids identified in pools of endometrial tissue obtained from endometrial samples collected 24 h after the last day of dietary supplementation, on D6, D14, and D22 from expected estrus of Nelore cows fed 1.7 kg/animal/day of the diet supplemented with sunflower seed or standard diet. It used 5 cows per timing group, totaling 15 cows per dietary treatment

Fatty acid	Abbreviation	Control group (%)	Sunflower group (%)	p-value
Short-chain fatty acids				
Hexanoic acid	C6:0	0.02±0.005	0.05±0.03	0.54
Octanoic acid	C8:0	0.02±0.002	0.03±0.02	0.42
Decanoic acid	C10:0	0.03±0.004	0.07±0.04	0.38
Decenoic acid	C10:1	0.02±0.002^{y}	0.03±0.002^{x}	0.09
Medium-chain fatty acids				
Dodecanoic acid	C12:0	0.03±0.03	0.08±0.03	0.35
Tetradecanoic acid	C14:0	0.94±0.02	1.2±0.25	0.34
cis-9-Tetradecenoic acid	C14:1 C9	0.02±0.002	0.07±0.04	0.11
Pentadecanoic acid	C15:0	0.35±0.02	0.36±0.02	0.76
Isopentadecanoic acid	C15:0 ISO	0.35±0.02^{a}	0.29±0.02^{b}	0.05
Anteisopentadecanoic acid	C15:0 ANTEISO	0.33±0.02	0.28±0.02	0.20
Hexadecanoic acid	C16:0	16.34±0.56	17.02±0.56	0.44
Isohexadecanoic acid	C16:0 ISO	0.23±0.05	0.16±0.05	0.36
cis-9-Hexadecenoic acid	C16:1 C9	0.65±0.05	0.64±0.05	0.90
Long-chain fatty acids				
Heptadecanoic acid	C17:0	0.73±0.03	0.65±0.03	0.16
Isoheptadecanoic acid	C17:0 ISO	0.14±0.01	0.15±0.01	0.66
Heptadecenoic acid	C17:1	0.17±0.05	0.21±0.05	0.61
Octadecanoic acid	C18:0	19.82±0.61	19.38±0.61	0.64
trans-6,7,8,9-Octadecenoic acid	C18:1 T6-T7-T8-T9	0.11±0.03	0.17±0.03	0.22
trans-10,11,12-Octadecenoic acid	C18:1 T10-T11-T12	0.59±0.09^{b}	1.13±0.09^{a}	0.01
cis-9-Octadecenoic acid	C18:1 C9	23.82±0.72	24.22±0.72	0.72
cis-11-Octadecenoic acid	C18:1 C11	2.64±0.15	2.65±0.15	0.98
cis-12-Octadecenoic acid	C18:1 C12	0.65±0.03	0.71±0.03	0.20
cis-13-Octadecenoic acid	C18:1 C13	0.11±0.02	0.17±0.02	0.18
cis-15-Octadecenoic acid	C18:1 C15	0.04±0.01	0.03±0.01	0.46
cis-9-trans-11-Octadienoic acid	C18:2 C9-T11	0.15±0.03	0.19±0.03	0.36
cis-9,12-Octadienoic acid	C18:2 C9-C12	6.29±0.46	6.56±0.46	0.69
n3-Octatrienoic acid	C18:3 n3	0.30±0.03	0.26±0.03	0.38
n6-Octatrienoic acid	C18:3 n6	0.17±0.09	0.22±0.09	0.68
Eicosanoic acid	C20:0	0.28±0.01	0.29±0.01	0.60
Eicosenoic acid	C20:1	0.53±0.04	0.55±0.04	0.82
Eicosadienoic acid	C20:2	0.36±0.05	0.44±0.05	0.27
n3-Eicosatrienoic acid	C20:3n3	0.58±0.03^{x}	0.45±0.03^{y}	0.05
n6-Eicosatrienoic acid	C20:3n6	2.11±0.22	1.88±0.22	0.51
Eicosatetraenoic acid	C20:4	10.92±0.74	10.57±0.74	0.76
Eicosapentaenoic acid	C20:5	0.57±0.04^{a}	0.39±0.04^{b}	0.04
Docosanoic acid	C22:0	0.12±0.02	0.09±0.02	0.33
Docosenoic acid	C22:1	0.08±0.01	0.05±0.01	0.21
Docosadienoic acid	C22:2	0.05±0.02	0.04±0.02	0.86
Docosapentaenoic acid	C22:5	3.90±0.16^{x}	3.35±0.16^{y}	0.08
Docosahexaenoic acid	C22:6	3.44±0.26	2.85±0.26	0.18
Tricosanoic acid	C23:0	0.07±0.001^{x}	0.04±0.01^{y}	0.08
Tetracosananoic acid	C24:0	0.08±0.01^{x}	0.05±0.01^{y}	0.08
Tetracosenoic acid	C24:1	0.79±0.27	1.31±0.27	0.24
Relative abundance of fatty acids in endometrial tissue (%)	98.94	99.32		

$^{a\ b}$Different letters in the same row indicate that the groups differ significantly ($p \leq 0.05$): ^{x,y}Different letters in the same line indicate a tendency to significance ($p \leq 0.1$)

Discussion

In this study, we observed that a diet supplemented with sunflower seed altered the bloodstream and endometrial tissue's lipid profile in female Nelore cows supplemented after estrus (i.e., early and late diestrus). This finding corroborates a previous study reporting that diets with a high-fat proportion stimulate the synthesis and accumulation of cholesterol (HDL predominance) in the tissues and body fluids of female cattle (Grummer and Carroll, 1988). Moreover, similarly, to our previous study (Cordeiro *et al*., 2015), a diet supplemented with sunflower seed increased total cholesterol, HDL, and LDL plasma concentrations.

In horses and ruminants, HDL is the primary lipoprotein that carries cholesterol to steroidogenic tissues, including the liver, ovaries, adrenal, and testicles, for membrane synthesis (Fernandes and Madureira, 2013). Indeed, we observed a gradual increase from D10 onward in plasma HDL concentrations of treated females. Moreover, we observed a gradual increase in LDL plasma concentration, but only at D21. Interestingly, a similar dynamic change was observed for cholesterol. The majority of blood cholesterol is transported through HDL and LDL (Grummer and Carroll, 1988; Bauchart, 1993; Kaneko *et al*., 2008). Therefore, it is expected that increases in plasma lipoprotein concentrations to be directly related to increases in cholesterol levels, as observed in the present study.

Our data showed that bovine females supplemented with sunflower seed had increased plasma cholesterol concentrations from D18 of supplementation onward. This supports previous findings that diets with high levels of lipids -such as sunflower seed, soybeans and tallow- increase blood cholesterol in beef cattle (Rafalowski and Park, 1982; Schauff *et al*., 1992; Cordeiro *et al*., 2015). However, cholesterol levels in cows have the potential to vary depending on a multitude of physiological factors, such as pregnancy and lactation and low cholesterol levels may have a detrimental impact on reproductive performance (Guedon *et al*., 1999).

Previous studies indicated that fat sources such as protected fat, fish meal, fish oil, flaxseed, and soybeans in the diet of beef or dairy cows may change the lipid composition of the uterus (Thatcher *et al*., 1997; Mattos *et al*., 2000; 2002; 2004; Robinson *et al*., 2002; Petit *et al*., 2004; Lopes *et al*., 2009). Indeed, this can occur because the change in the profile of fatty acids absorbed through the small intestines alters the fatty acids profile in the blood, as well as in the reproductive tissues of cattle (Santos *et al*., 2008; Cooke *et al*., 2014; Cipriano *et al*., 2016). Therefore, PUFA supplementation alters the physiology and potentially improves the cows' reproductive performance through changes in the fatty acids profile of reproductive tissues (Santos *et al*., 2008). Specifically, PUFA supplementation in diet changes the lipid composition of the endometrium by making it less luteolytic (Mattos *et al*., 2000).

In the present study, sunflower seed supplementation to beef cows altered the endometrial fatty acids profile, with notable changes among long-chain fatty acids. Interestingly, diets composed of long-chain fatty acids can decrease PGF2α secretion and have an anti-luteolytic effect, which can impact the interaction between the uterus and conceptus (Thatcher *et al*., 2011).

Specifically, we also observed that the relative abundances of n3-Eicosatrienoic (C20:3 n3), Docosapentaenoic (C22:5), and Eicosapentaenoic (EPA; C20:5) unsaturated fatty acids in the endometrium were higher in control than in the treated group. Interestingly, EPA (C20:5) is a precursor of prostaglandin-F3α (PGF3α), which is a product of linolenic acid conversion. Moreover, increased linolenic acid in the diet can decrease the production of dienoic prostaglandins such as PGF2α (Wathes *et al*., 2007).

Surprisingly, we observed a lower EPA relative abundance in the endometrial tissue of treated females. Regardless, there were no differences in linoleic acid (cis-9,12-octadienic), conjugated linoleic acid (C18:2 C9-T11), and DHA relative abundances in the control compared to the treated group. It should be noted, however, that the main limitation of the present study was our inability to predict the arrival of specific lipids, particularly PUFA, to the small intestines for absorption. Additionally, we were not able to predict the fatty acid demand of different reproductive tissues.

Similar to supplementation with a sunflower seed in the present study or protected n-6 acids (Cheng *et al*., 2001), abomasal infusion of fat containing a high amount of linoleic acid resulted in less PGF2α release on D15 in response to oxytocin (Oldick *et al*., 1997). Thus, it can be speculated that sunflower seed may reduce the synthesis and release of endometrial PGF2α, which would benefit pregnancy establishment in beef cattle. Nevertheless, the mechanism of action of fats-via energy increase or PUFA supply needs to be further investigated.

Conclusion

The present study has led us to conclude that a diet enriched with sunflower seed alters the lipid composition of plasma and endometrium in female beef cattle. Our findings suggest that lipid changes in the plasma and endometrium of beef cattle may be associated with reduced embryonic mortality and, consequently, with an increase in conception rate in beef cows. The conception rate may increase by about 19% after sunflower seed supplementation in beef cows subjected to TAI or TET.

Then, it is estimated that, for a herd of 100 cows, the annual increase in production is 19 calves. In 2022, the cost per calf in Brazil had an average price of R$ 2,600 (~USD 500). Thus, the use of a diet containing sunflower seed may represent an annual gain of R$ 49,400 (~USD 9,500) per 100 cows. Therefore, sunflower seed supplementation may be a positive cost/benefit relationship in TAI and TET programs.

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Author's Contributions

Mariângela Bueno Cordeiro Maldonado: Responsible for performing and overseeing the entire experimental phase, including data collection, conducted the study, prepared the manuscript, conducted literature research, and performed the final revision of the study.

Ricardo de Oliveira Rodrigues: Responsible for the development of the entire experimental part and data collection.

Dante Pazzanese Duarte Lanna: Responsible for fatty acids profile analysis by gas chromatography, interpretation of data, and provided final approval for the manuscript's version.

Guilherme Pugliesi and Ricardo da Fonseca: Responsible for data analysis, interpreted the found, conducted literature research, and performed the final revision of the study

Milton Maturana Filho: Responsible for plasma lipid composition analysis by enzymatic method, interpretation of data, and provided final approval for the manuscript's version.

Adriano Felipe Mendes and Lucas de Oliveira Bezerra: Responsible for the development of the experimental phase and undertaken data collection responsibilities.

Claudia Maria Bertan Membrive: Project coordinator, oversaw responsibilities such as experimental design, study writing, manuscript preparation, data analysis and interpretation, literature search, and final study revision.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that no ethical issues are involved.

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