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Feeding Calcium Salts of Linseed Oil on Metabolic Status and Reproductive Traits in Grazing Dairy Cows

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Abstract

This experiment aimed to evaluate the effect of calcium salts of linseed oil (rich in alpha-linolenic acid (ALA, C18:3n-3) on metabolic and reproductive traits in high-producing dairy cows under grazing. Thirty-six Holstein dairy cows were randomly assigned, in a complete block design, to receive ALA supplementation (0.85 kg·day^{-1} of calcium salts of linseed oil) or to remain as untreated control (CON). The concentrate was formulated to offer the same amount of energy across treatments (CON cows received an extra kg of corn to compensate for the higher energy density of ALA treatment). A PMR + Alfalfa pasture was offered to all cows at the same time. A fixed time artificial insemination (FTAI) at 80 DIM, preceded by a Presynch plus Ovsynch protocol was implemented for the first service and later, on return to estrus, heat detection and artificial insemination (AI) were performed. Pregnancy diagnosis was checked at 30, 42, 60, and 90 d after AI. Blood and milk samples were taken biweekly. Treatment affected plasma cholesterol concentration (160.36 vs. 186.70 mg·dl^{-1}, $p = 0.03$, for ALA and CON, respectively) and on size of corpus luteum (CL, 17.6 vs. 13.7 mm, $p = 0.02$, for ALA and CON, respectively). Supplementation tended ($p = 0.136$) to increase conception rate by 200 DIM (81.69% vs. 55.43% in ALA and CON cows, respectively). However, treatment had no effect ($p > 0.05$) on body weight (BW), body condition score (BCS), and circulating levels of beta-hydroxybutyrate (BHBA), glucose, insulin, growth hormone (GH) and insulin-like growth factor (IGF-I). Our results suggest that supplementation with calcium salts of linseed oil could enhance ovarian function without affecting energy metabolism in early lactation dairy cows.

Keywords

α-Linolenic Acid, Supplementation, Reproduction, Grazing, Dairy Cows

1. Introduction

Subfertility, sometimes associated with negative energy balance (NEB), has a detrimental impact on the productivity of dairy systems. Lipid supplementation in early lactation may partially attenuate the NEB; however, the positive influence of lipid supplementation on reproduction is independent of the cow's energy balance [1]. [2] showed that the type of fatty acid (FA) that is offered to animals through the diet, and not the addition of extra energy, stimulates the ovary and generates the development of follicular structures of large size. In this regard, it has been suggested that long-chain dietary FA, particularly polyunsaturated FA (PUFA), can improve the reproductive performance of dairy cattle.

There are two main families of essential PUFA called omega-3 and omega-6 that could affect fertility. Omega-3 is a precursor of the trienoic (3 series) prostaglandins and omega-6 of the dienoic (2 series) prostaglandins. The main source of omega-6 FA is linoleic acid from the diet (C18:2n-6), predominant in sunflower and soybean oils, and can be converted to arachidonic acid (C20:4 n-6), which is a precursor of dienoic prostaglandin (PG), such as PGF2*α*. The same elongase and desaturase enzymes, also convert the main omega-3 FA of the diet (*α*-linolenic acid; C18:3n-3), abundant in linseed oil, to eicosapentaenoic acid (EPA; C20:5 n-3), which is a precursor of the trienoic PG, such as the PGF3*α* [3] that has less biological activity on the corpus luteum (CL). There is an enzymatic competition between omega-3 and omega-6 for the desaturation and elongation, as well as for the PG synthetase site. The composition of phospholipids in cell membranes is related to the lipids from the diet, which determines the amount and type of prostaglandins synthesized from them [4] [5]. Therefore, an increase in the supply of omega-3 favors the synthesis of PGF3*α* instead of PGF2*α* in endometrial cells and contributes to a reduction in embryonic mortality [6] [7] by reducing luteolytic activity.

Most embryonic losses in cattle occur between 8 and 16 days after AI [8] due to some embryos may not reach a sufficient size at that time to inhibit the synthesis of PGF2*α* and, therefore, luteolysis ensues [9]. The loss of embryos in such cases may be associated with their inability to inhibit the luteolytic action of PGF2*α* during the critical period of maternal recognition of pregnancy [10]. In this context, it's proposed that the inhibition of PGF2*α* synthesis could increase embryonic survival rate and pregnancy rate [11].

On the other hand, diet lipids could stimulate ovarian activity by improving follicular development, increasing progesterone production, and/or decreasing progesterone *clearance,* which could lead to higher pregnancy rates [12].

Even though the supply of omega-3 FA has a significant positive effect on the

reproductive performance of dairy cows in intensive production systems (100% TMR diets), studies referring to the effect of omega-3 FA on reproductive and fertility indicators in pasture-based production systems are very scarce [13].

This experiment aimed to evaluate the effects of C18:3n-3 supplementation on energy balance and ovarian dynamics in early lactation-grazing dairy cows. We hypothesized that the supplementation with rumen-protected FA rich in C18:3n-3 improves ovarian function without affecting energy balance in grazing dairy cows.

2. Material and Methods

2.1. Animals and Treatment

The experiment took place at the National Institute of Agricultural Technology (INTA) Rafaela Experimental Farm located in the main dairy region of Santa Fe, Argentina (31˚12'S, 61˚30'W) for 12 weeks (2 weeks for diet adaptation and 10 weeks for data collection). All procedures were approved by the Welfare Committee of La Plata National University (Buenos Aires, Argentina). Holstein cows (n:36) 58.0 ± 17.0 DIM, having a body weight (BW) of 594.1 ± 92.4 kg and abody conditional score (BCS) of 2.71 ± 0.39, with 2.6 ± 1.5 parities and producing 38.9 ± 9.3 kg·day^{-1} were blocked (18 blocks) and randomly assigned to receive alpha-linolenic acid (ALA) or to remain as untreated control (CON). Lipid supplementation started 4 weeks before the first AI and lasted for 12 weeks (end of study). During the 3 weeks before the start of the trial (covariate), the cows received the control diet. All cows were equipped with neck transponders that served both to record daily milk production and allocate concentrate on an individual basis in the milking parlor (ALPRO version 6.60/DeLaval, Tumba, Sweden).

Rations were formulated to meet the requirement of a 600 kg Holstein dairy cow, 60 DIM, producing 42.0 kg·day^{-1} of milk with 3.7% fat [14]. Two diets were formulated for the study: ALA diet contained 0.85 kg·day^{-1} of calcium salt of linseed oil (82.3% dry matter (%DM), 13.2% ash, 86.8% ether extract, and 36.0% of C18:3n-3) per cow whereas CON diet was formulated to offer an extra amount of cracked corn grain to compensate the total energy offered in ALA. Cows received individually a mixture of commercial feed (3.62 kg DM/cow/d; both groups), cracked corn (0.88 and 2.30 kg of DM/cow/d for ALA and CON respectively), and calcium salt of linseed oil (0.70 kg DM/cow/d; only cows at ALA) at milking. All cows received the same management and were fed together, but differentially supplemented at milking time. After milking (2:30 a.m. and 1:30 p.m.), cows were housed in a dry lot for PMR supply (PMR; composed of corn silage, soybean meal, ground corn, and alfalfa hay). Once cows finished PMR consumption, they were conducted to the alfalfa pasture until the a.m. milking (2:30 p.m.). Alfalfa (*Medicago sativa*) pasture was offered at a daily rate of 12 kg DM per cow and PMR at a daily rate of 13.5 kg DM per cow. The ingredients and nutrients of the experimental diets are presented in **Table 1**.

Table 1. Ingredient and nutrient composition of the experimental diets with calcium salts of linseed oil (ALA) or not (CON).

Item	Dietary treatment	
	ALA	CON
Ingredient, kg·DM·cow^{-1}·day^{-1}		
Alfalfa	8.00	8.00
Corn silage	5.88	5.88
Soybean meal	2.71	2.71
Ground corn	2.76	2.76
Alfalfa hay	1.33	1.33
Commercial feed	3.62	3.62
Cracked corn	0.88	2.30
AG-Ca	0.70	0.00
Nutrient		
DM (%)	40.38	41.02
CP (% of DM)	18.31	18.26
NDF (% of DM)	24.35	24.22
ADF (% of DM)	13.48	13.28
ADL (% of DM)	2.36	2.35
EE (% of DM)	6.61	4.48
Ash (% of DM)	7.57	7.13
ME[a] (Mcal·kg·DM^{-1})	2.79	2.71

Abbreviations: AG-Ca, calcium salts of linseed oil; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; EE, ether extract; ME, metabolizable energy. [a]ME was estimated according to NRC (2001).

2.2. Feed Analysis

Representative samples of concentrate, total PMR, PMR ingredients, and pasture were taken every 10 days. Pasture samples were obtained manually in the grazing horizon simulating the selectivity of the cow (hand-plucking) [15]. All samples were oven-dried with forced air circulation at 65˚C and 105˚C to constant weight to determine the DM content and ground in a Wiley mill (1 mm mesh). The content of ashes (AOAC 1990, procedure 942.05), crude protein (CP) (AOAC 1998, procedure 976.05), neutral detergent fiber (NDF) (ANKOM Technology Method 6-2011 validated with NFTA protocol), acid detergent fiber (ADF) (ANKOM Technology Method 5-2011 validated by AOAC 1990, procedure 973.18), acid detergent lignin (ADL) (PROMEFA protocol validated for ANKOM equipment) and ether extract (EE) (AOAC 1998, procedure 920.39, modification for automatization extract) was determined. Lipid metabolizable energy (ME) content (6.50 Mcal·kg·DM^{-1}) was calculated based on the equations from [14].

2.3. Reproductive Management

All cows were examined after parturition once a week to make sure they were in optimal condition at the beginning of the service. Cows received a fixed time artificial insemination (FTAI) at 81 ± 21 and 80 ± 19 DIM for ALA and CON with a Presynch plus Ovsynch protocol for the first service and later, on return to estrus, heat detection, and artificial insemination (HD-AI) AM/PM. Pregnancy diagnosis was evaluated by ultrasonography on days 30, 42, 60, and 90 post-AI. Nonpregnant cows were detected in estrus and AI. Briefly, Presynch consisted of 2 IM doses of 25 mg of $PGF_{2\alpha}$ (Lutalyse, ZOETIS; 10 Sylvan Way Parsippany, NJ 07054, EEUU) separated by 14 days. Ovsynch protocol started 12 days after the second dose of $PGF_{2\alpha}$ with the IM application of 10 μg of GnRH (Buserelin Acetate, Biosin, BIOTAY; Rutherford 4503 - B1615GYC - Grand Bourg, Buenos Aires, Argentina), 7 and 8 days later an IM injection of $PGF_{2\alpha}$, on day 9 an IM dose of GnRH and 16 h later a FTAI was implemented.

2.4. Sampling and Measurements

Cows were individually weighed with an electronic scale after the morning milking every week and preventing access to water about 2 hours before weighing. At the same time, BCS was determined by two independent observers by using a 5-point scale (1 = extremely thin and 5 = extremely fat) with 0.25 increments [16] and the value analyzed was the average result of both evaluators.

Daily milk production was measured individually with the milk measurement system DeLaval ALPRO (DeLaval International AB, Tumba, Sweden), considering averages by week. Milk composition was evaluated from individual samples collected weekly. Two milk subsamples were taken from each cow in consecutive milkings by using milk meters (DeLaval International AB, Tumba, Sweden), then a single individual sample (pool) weighted by the respective production was obtained. In each composite sample the content of fat, total protein, and lactose was determined by infrared spectrophotometry (MilkoScan™ Minor; FOSS Electric, Hilleroed, Denmark) according to the standard method ISO 9622 IDF 141 (2013). Energy-corrected milk (ECM) was calculated according to [17].

Dry matter intake (DMI) was measured individually at the end of the study. Total DM intake was estimated by an equation used by [14] whilst pasture DM intake was estimated by subtracting PMR and concentrate DM intakes (determined by the difference method) from total DM intake. The estimation of total DMI was calculated *post hoc* based on milk production level and individually per cow as [18] and [19] described in previous studies.

Blood samples were obtained every 2 weeks by jugular venipuncture after the morning milking. Blood was collected in tubes containing sodium heparin (5 U/ml). Plasma was obtained by centrifugation (2000 ×g for 15 min at 4˚C) and stored at −24˚C until glucose analysis (Enzymatic glycemia, Wiener Laboratory, Rosario, Argentina), urea (Uremia, Wiener Laboratory, Rosario, Argentina), total cholesterol (Enzymatic colestat, Wiener Laboratory, Rosario, Argentina), in-

sulin, growth hormone (GH) and insulin-like growth factor (IGF-I) as described in [20]. Beta-hydroxybutyrate (βHBA) was determined in whole blood with a Free Style Optium ketone test (Abbot Diabetes Care Ltd., Witney, UK).

Ovarian activity was assessed by transrectal ultrasonography (US) with a portable ultrasound Aquila Pro (Pie Medical) B mode, in real-time and with a lineal transducer of 8 MHz to determine the number and size of ovarian structures (follicles [FOL] and corpus luteum [CL]). The day of FTAI was declared as day 0. Two randomly selected subgroups of 9 cows from ALA and CON were monitored daily from the first application of GnRH (day -10) up to day 1 (ovulation diagnosis) and then on day 6 to verify the presence of a CL on the same ovary where ovulation occurred. The other cows were tested on specific days (−10, −3, −2, −1, 0, 1, and 6). The size of ovarian structures was calculated by the average of the 2 perpendicular diameters. Cavity of CL was calculated in the same way and subtracted from the total size. Blood samples for progesterone measurements were collected on days 12, 19, and 21 by coccygeal venipuncture. The samples were centrifuged (2000 ×g for 15 min at 4˚C) and the serum obtained was stored at −18˚C until analyzed (Radioimmunoassay, Instituto de Biología y Medicina Experimental, Buenos Aires).

2.5. Study Design and Statistical Analysis

A randomized complete block design was used with the cow as the experimental unit. Entry to the study was the blocking criterion given that cows were matched by parity, milk yield, DIM, BCS, and BW. The effect of ALA supplementation on indicators of metabolic status, ovarian activity, and progesterone concentration was assessed by linear mixed models with repeated measures by using the PROC MIXED of SAS (SAS/STAT ver. 9.4, SAS Institute Inc., Cary, NC). Statistical significance was set at $p < 0.05$ and a tendency was declared at $p \leq 0.10$. The models included the random effect of the cow nested to block and the fixed effects of time (as sampling weeks [0 vs. 4 vs. 6 vs. 8 vs. 10 vs. 12] when assessing metabolic status, and as sampling days [−5 vs. −4 vs. −3 vs. −2 vs. −1 vs. 0] when assessing ovarian activity), treatment (ALA vs. CON), and their interaction. The covariance structure having the smallest Akaike information criterion and Schwarz's Bayesian criterion was used [21]. Time was removed from the model assessing the effect of ALA supplementation on DMI and the size of FOL and CL at days 0 and 6, respectively. Finally, the effect of ALA supplementation on conception at TAI (PRTAI) and on conception by 200 DIM (PR200) was assessed by logistic models by using PROC GLIMMIX of SAS with binomial distribution and logit link function. Models included the fixed effect of treatment (ALA vs. CON) and were controlled by DIM at TAI.

3. Results

Treatment did not affect total DMI (22.7 vs. 22.1 kg·DM·day^{-1} in ALA and CON cows, $p = 0.61$, **Figure 1**). However, treatment had an effect on concentrate in-

take given that ALA consumed less than CON (3.9 vs. 5.9 kg·DM·day^{-1}, respectively, $p < 0.01$, Figure 1). Treatment tended to affect the intake of pasture given that ALA consumed more than control (5.6 vs. 3.4 kg·DM·day^{-1}, $p = 0.06$, Figure 1) and the intake of PMR (13.1 vs. 12.8 kg·DM·day^{-1} for ALA and CON respectively, $p = 0.08$, Figure 1). Treatment did not affect total ME intake (62.6 vs. 60.8 Mcal·day^{-1} in ALA and CON cows, $p = 0.44$, Figure 1), but it had a trend to affect the intake of ME from pasture (14.6 vs. 9.3 kg·Mcal·day^{-1} for ALA and CON respectively, $p = 0.06$, Figure 1), the intake of ME from concentrate (13.7 vs. 18.0 kg·Mcal·day^{-1} for ALA and CON respectively, $p < 0.01$, Figure 1) and, also, the intake of ME from PMR given that ALA consumed more than CON (34.3 vs. 33.4 kg·DM·day^{-1}, $p = 0.08$, Figure 1).

Treatment did not affect milk production (33.4 vs. 33.8 kg·day^{-1} in ALA vs. CON cows, $p = 0.72$). Regarding milk fatty acids composition, milk from cows supplemented with calcium salts of linseed oil had practically two times the concentration of *α*-linolenic-acid (ALA, C18:3n-3) to control group (1.0 vs. 0.48 g/100 g of FA; $p < 0.01$). This difference could be explained by the supplementation and may demonstrate C18:3n-3 intestinal absorption. For further details see González *et al.* (2020).

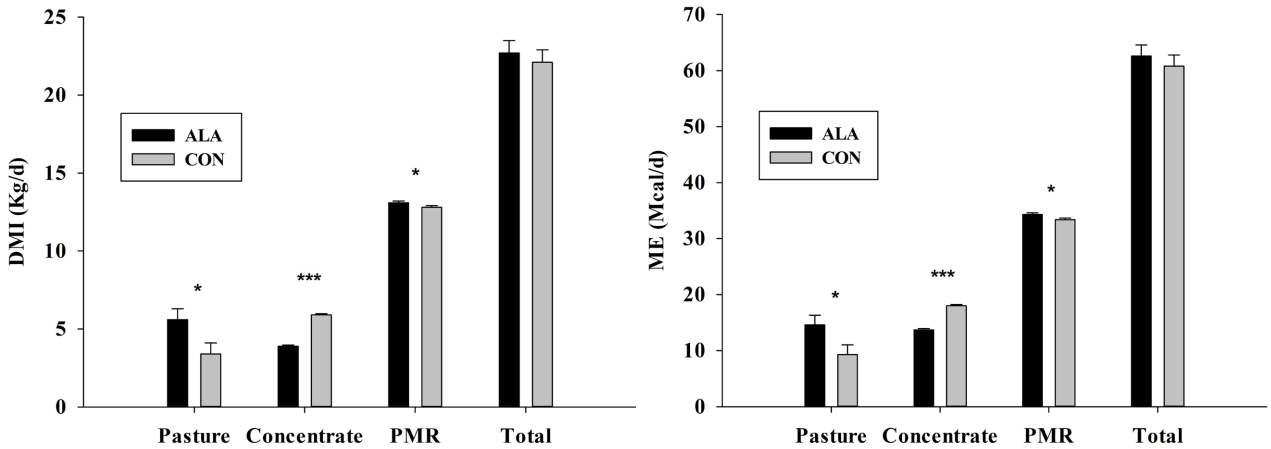

Figure 1. Dry matter intake and metabolize energy intake in dairy cows supplementing with calcium salts of linseed oil (ALA) or not (CON). Values are expressed as the least squares means and standard error (SEM). ME is estimated according to NRC (2001); values of ME for pasture, ALA: concentrate, CON: concentrate and PMR: 2.61, 3.50, 3.05 y 2.61 Mcal·kg·DM^{-1}, respectively. *$p < 0.10$, **$p < 0.05$, ***$p < 0.01$.

3.1. BW, BCS, Metabolites and Hormones

The effects on variables associated with body reserves mobilization and plasma concentration of metabolites and hormones are shown in Table 2. Time had a significant effect on all the measured variables ($p < 0.01$), but a tendency for BHBA ($p = 0.07$, Table 2). Treatment had only an effect on cholesterol and urea, given that cholesterol concentrations were 186.70 and 160.36 for ALA and CON cows ($p = 0.03$, Table 2) and, urea concentrations were 0.42 and 0.40 for ALA

and CON cows ($p = 0.01$, Table 2). Also, time by treatment interaction affected cholesterol, given that ALA had higher concentrations than CON from weeks 4 to 10 (Figure 2; $p = 0.009$). Conversely, treatment did not affect BW (581.5 and 578.7 for ALA and CON cows, $p = 0.503$, Table 2), BCS (2.74 and 2.66 for ALA and CON cows, $p = 0.23$, Table 2), blood glucose concentration (0.60 in ALA and CON cows, $p = 0.62$, Table 2), and BHBA (0.57 in ALA and 0.57 in CON cows, $p = 0.53$, Table 2). Treatment did not affect insulin (0.91 in ALA and 0.95 in CON cows, $p = 0.36$, Table 2), GH (2.42 in ALA and 2.45 in CON cows, $p = 0.94$, Table 2), IGF1 (109.37 in ALA and 109.79 in CON cows, $p = 0.49$, Table 2) and GH:Insulin ratio (3.08 in ALA and 2.46 in CON cows, $p = 0.19$, Table 2). When looking overall, only plasma cholesterol and urea were affected by treatment, being in both cases higher for ALA compared to CON ($p = 0.030$ and 0.012 for cholesterol and urea respectively; Table 2 and Figure 2).

Table 2. Mixed model effects on indicators of metabolic status in Holstein Dairy Cows supplemented with calcium salts of linseed oil (ALA) and without calcium salts of linseed oil (CON) (n: 36).

Trait	Treatment			Effects		
	CON	ALA		Treatment	Time[a]	Treatment* Time
	(LSM)	(LSM)	(SEM)		*p*	
BW	578.7	581.5	2.88	0.503	<0.001	0.521
BCS	2.66	2.74	0.04	0.236	<0.001	0.533
Glucose ($g \cdot l^{-1}$)	0.60	0.60	0.01	0.616	<0.001	0.657
BHBA ($mmol \cdot l^{-1}$)	0.57	0.59	0.02	0.530	0.075	0.970
Cholesterol ($mg \cdot dl^{-1}$)	160.36	186.70	7.50	0.030	<0.001	0.009
Urea ($g \cdot l^{-1}$)	0.40	0.42	0.01	0.012	<0.001	0.345
Insulin ($ng \cdot ml^{-1}$)	0.95	0.91	0.03	0.358	<0.001	0.852
GH ($ng \cdot ml^{-1}$)	2.40	2.42	0.20	0.942	<0.001	0.943
IGF-1 ($ng \cdot ml^{-1}$)	109.79	109.37	9.09	0.493	<0.001	0.868
GH:Insulin ratio	2.46	3.08	0.32	0.196	0.060	0.794

Abbreviations: BW, body weight (kg); BCS, body condition score (5-point scale); BHBA, beta-Hydroxybutyric acid; GH, growth hormone; IGF-1, insulin-like growth factor 1. [a]Time: days of supplementation period (blood sampling week: 4, 6, 8, 10, 12).

3.2. Ovarian Structures and Plasma Progesterone

At the beginning of the Ovsynch protocol, after Presynch, 72.2% and 75% presented CL in ALA and CON groups, respectively. Treatment did not affect the size of preovulatory dominant FOL ($p > 0.1$, Table 3). Treatment did not affect the number of follicles (0.59 vs. 0.80 in ALA and CON, respectively, $p = 0.35$). Treatment increased the size of CL at day 6 given that ALA cows had 3.9 mm greater CL than CON cows (17.6 vs. 13.7 mm, $p = 0.02$, Table 3). Treatment did not affect progesterone concentration on days 12 and 19 ($p > 0.1$, Table 3), but

conversely, it increased concentration on day 21 compared with CON (2.21 vs. 1.06 ng·ml^{-1}, $p < 0.01$, Table 3).

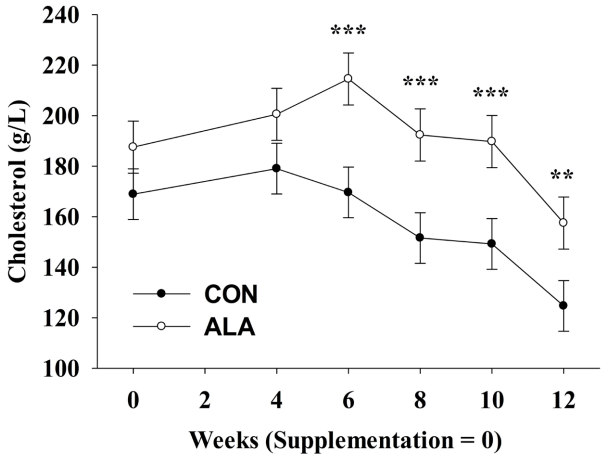

Figure 2. Linear mixed model effect of supplementation with unsaturated fatty acids on cholesterol concentration in Holstein Dairy Cows (n: 36). *$p < 0.10$, **$p < 0.05$, ***$p < 0.01$.

Table 3. Effect of calcium salts of linseed oil (ALA) supplementation on ovarian activity and plasma progesterone in early lactation grazed dairy cows (n = 38).

	CON	ALA		
	(LSM)	(LSM)	(SEM)	*p*
Number of follicles per ovary	0.80	0.59	0.15	0.35
Size of preovulatory follicles (mm)	16.7	16.3	0.7	0.71
Size of CL at day 6 (mm)	13.7	17.6	1.2	0.02
Progesterone at day 12 (ng·ml^{-1})	0.75	0.98	0.27	0.55
Progesterone at day 19 (ng·ml^{-1})	1.37	1.31	0.28	0.87
Progesterone at day 21 (ng·ml^{-1})	1.06	2.21	0.30	<0.01

Abbreviations: FOL, follicle; CL, corpus luteum; day 6, 19 and 12, are days from fixed timed artificial insemination.

3.3. Fertility

Treatment did not significantly improve conception rates at FTAI (62.65% vs. 52.10% in ALA and CON cows, respectively [OR = 1.542, 95% CI = 0.328 - 7.248, $p = 0.573$]). But it tended to increase conception rate by 200 DIM in 26.3 percentage points (81.69% vs. 55.43% in ALA and CON cows, respectively [OR = 3.587, 95% CI = 0.653 - 19.718, $p = 0.136$]).

4. Discussion

Our findings that supplementation with protected linseed oil in grazing dairy increases the size of CL, and blood progesterone concentration without affecting BW, BCS, glucose, BHBA, insulin, GH and IGF1, total DMI, total ME intake,

and milk production supports our working hypothesis stating that rumen protected FA rich in C18:3n-3 improve ovarian function without affecting energy balance in grazing dairy cows. In this experiment, there was no effect on the total DMI and ME intake in cows supplemented with ALA, which may explain the absence of differences in milk production between treatments.

It is known that hormones may play an important role in coordinating the partition of dietary fatty acids between milk fat secretion, deposition in adipose tissue, and body lipid mobilization. Some hormones like GH, IGF-1, and insulin, are crucial when evaluating energy balance. At the time of the experiment, cows were in 58.0 ± 17.0 DIM, so we considered that the concentrations of these hormones should be within the normal range and treatment would not have a major effect on them. There are also no differences in BCS that suggest a different anabolic state between treatments.

The reduction of starch consumption in the ALA group could lead to a reduction in glycemia and insulinemia, but this did not happen in this trial. Blood samples were taken after ingestion of the supplement during milking time, but no differences were observed in these two parameters. The explanation for this may be that fat supplementation reduces glucose oxidation [22].

Plasmatic metabolites such as βHBA and glucose and regulatory hormones such as insulin, GH, and IGF-I act as dynamic or short-term indicators of energy balance [23]. Cows in a negative energy balance show a decrease in plasmatic glucose, insulin, and IGF-I concentrations and an increase in βHBA and GH levels [24]. Taken together, the results obtained in this study (similar plasma concentrations of βHBA, glucose, insulin, GH, and IGF-I) suggest that supplementation with rumen-protected linseed oil in replacement of corn as an energy source did not affect the energy metabolism of dairy cows in early lactation. This lack of ALA effect on βHBA was in accordance with the absence of variations in metabolic parameters and with a similar GH/insulin ratio in both groups (**Table 2**). In this sense, [24], suggested that the hormonal profile may play a more important role than the type of diet (fat) in regulating body lipid mobilization in the early-lactation dairy cow.

In cattle, GH is the main hormone stimulating milk production through direct and indirect mechanisms (IGF system and interactions with insulin) [25]. Hepatic production of IGF-I is positively correlated with both, energy intake and plasma GH and insulin levels [26]. The lack of a significant effect of fat on IGF-I concentrations in the present experiment is consistent with similar GH and insulin levels observed (**Table 2**) and with the similar ($p = 0.44$) energy intake of cows (**Figure 1**).

Even though the sizes of preovulatory FOL were not different between groups, the size of the main CL at day 6 was significantly greater in cows of the ALA group. This could be explained by some effect generated by lipids on the development of the CL acting on ovarian intrinsic or extrinsic factors. In the endometrium, gene expression associated with different patterns of prostaglandin synthesis is a consequence of the n-6: n-3 ratio influencing the luteal lifespan [5].

There is no evidence of any luteotropic influences between days 5 to 7 of gestation. However, bovine embryos generated an anti-luteolytic stimulus on cocultured luteal cells [27] and this could also explain the major sizes of CL reported in ALA treatment that had greater conception rates.

The greater size of the main CL together with the higher progesterone concentration in ALA cows would explain the finding that ALA tended to increase pregnancy rate at 200 DIM by 26.3 percentage points (81.69% vs. 55.43%). A greater mean size of the CL has been reported for cows receiving omega-3 FA in the form of either formaldehyde-treated linseed [28] or whole linseed [29] and is a factor that contributes to an increase in the plasmatic concentration of progesterone [30]. [6] also found no effect on follicular dynamics, but the CL was larger in cows fed whole flaxseed treated with formaldehyde or a mixture of fish oil and formaldehyde-treated whole flaxseed.

[1] proposed the hypothesis that dietary fats can modulate the survival of the CL by reducing PGF2α secretion to improve pregnancy. One study [31] indicates that omega-3 FA causes disruption in the lipid microdomain structure and alters lateral mobility of the PGF2α receptor in bovine luteal cells. This could alter luteal sensibility to PGF2α and may be the cause of the difference in fertility presented between treatments. Even when the ratio of omega-6: omega-3 supplied through the diet changes, the pattern of PGF2α secretion changes as well [5]. Finally, the change in the fatty acid profile of the diet modifies the fatty acid composition of the follicular fluid. [32] found that the proportion of ALA in the follicular fluid in cows fed encapsulated flaxseed oil was 2.5 times higher than in control cows fed encapsulated saturated fatty acids. Therefore, the fatty acid profile could play a key role in the lifespan of the CL acting through intrinsic factors of the ovary.

Additionally, the greater progesterone concentration observed in ALA cows at day 21 coincides with the greater proportion of pregnant cows and greater size of CL at day 6 in this group. Progesterone concentration could be explained by the higher levels of cholesterol detected in these cows, given that cholesterol is a precursor for the synthesis of progesterone in luteal cells [1]. The concentration of plasma cholesterol is increased consistently under regimens of supplemental fat [33]. [34] proposed that an increase in dietary lipids would stimulate intestinal biosynthesis of cholesterol to meet the increased demand for fat absorption and transport, which would help explain the higher plasma cholesterol concentration in ALA (**Figure 2**). In our study, the size of preovulatory FOL and the level of circulating IGF-1 are not affected by treatment, but, conversely, the size of CL at day 6 is (**Table 3**). These findings are good because it has been reported that low IGF-1 concentrations could lead to low steroidogenesis [35] and, that small CL with poor progesterone secretion could lead to small follicle size and poor conception rates [36]. Also, low progesterone concentration could partially be responsible for reduced fertility [30] and increased pregnancy loss [37] in dairy cows.

We also found that ALA supplementation did not decrease the lipid mobiliza-

tion and BW loss characteristic of early lactation cows [38]. Similar results were observed in early lactation grazing dairy cows [20] [39] [40].

Finally, it is known that ALA supplementation improves fertility by reducing endometrial inflammation. However, further research is needed to investigate how lipids participate in the formation and function of the CL to enhance conception rates. As pointed out by [41], the mechanisms through which fatty acids influence follicular development remain unknown.

5. Conclusion

As the supplementation with protected linseed oil in grazing dairy increases the size of CL, blood progesterone concentration, and it tended to increase conception rate by 200 DIM without affecting BW, BCS, glucose, BHBA, insulin, GH and IGF1, total DMI, total ME intake, and milk production we concluded that supplementation with rumen-protected FA rich in C18:3n-3 improves ovarian function without affecting energy balance in grazing dairy cows. The use of this supplement seems promissory; however, additional studies with greater numbers of animals are necessary to confirm its effect on reproductive performance.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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