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Effects of Feeding Calcium Salts from a Mixture of Linseed and Fish Oil on Productive Response, Metabolic Status, and Reproductive Parameters in Early-Lactation Dairy Cows

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Abstract

This study evaluated the effects of supplementing early-lactation Holstein cows with rumen-protected omega-3 fatty acids (calcium salts) on productive and reproductive performance. Thirty-six multiparous cows were randomly assigned to one of two treatments from 21 ± 2 days before calving to 105 ± 5 days in milk (DIM): a Control group (C) or an Omega-3-supplemented group (O-3), receiving a blend of linseed and fish oil (60:40). Both groups were fed isoenergetic diets, with ground corn as the control supplement. Total dry matter and net energy intake did not differ between treatments. A treatment-by-time interaction was observed for milk yield, with cows in the O-3 group producing more milk than controls at specific time points. Additionally, O-3 cows had higher overall protein yield and improved feed efficiency. No differences were found in body weight, BCS, glucose, insulin, IGF-1, or urea concentrations, but a tendency toward higher plasma NEFA and BHBA concentrations and lower energy balance was observed in the O-3 group. Supplementation increased plasma cholesterol and progesterone concentrations and was associated with a higher proportion of cows being pregnant at 130 DIM. These findings suggest that omega-3 supplementation may improve specific aspects of lactational performance and reproductive efficiency without compromising metabolic status.

Keywords: omega-3 fatty acids; dairy cows; transition period; linseed oil; fish oil

Received: 7 May 2025
Revised: 9 June 2025
Accepted: 11 June 2025
Published: 1 July 2025

Citation: Roskopf, P.M.; Cuatrin, A.; Stangaferro, M.; Storani, G.; Angeli, E.; Hein, G.J.; Salado, E.E. Effects of Feeding Calcium Salts from a Mixture of Linseed and Fish Oil on Productive Response, Metabolic Status, and Reproductive Parameters in Early-Lactation Dairy Cows. *Dairy* **2025**, *6*, 34. https://doi.org/10.3390/dairy6040034

1. Introduction

Dairy cows have to afford several endocrine, metabolic, reproductive, and immunological challenges during the productive cycle. These challenges are particularly pronounced during the transition period (TP), when cows experience a reduction in dry matter intake (DMI), leading to negative energy balance (NEB), lipomobilization, and body mass loss. This metabolic stress occurs alongside drastic changes in hormone concentrations, decreased blood calcium levels, and uterine contamination in the immediate postpartum

period [1,2]. These factors, combined with poor management practices, can promote immunosuppression, increase the risk of various diseases, and negatively affect productive and reproductive performance.

In this context, lipid supplementation can increase the energy density of diets [3]. However, the specific effects of lipid supplementation vary depending on the fatty acid (FA) profile of the lipid source [4,5]. Polyunsaturated fatty acids (PUFAs) of the omega-3 family are known to influence the expression of genes involved in various metabolic pathways [6,7]. PUFA supplementation, particularly with calcium salts rich in omega-3 fatty acids, has shown variable effects on milk yield but more consistent improvements in milk fat composition [8–11]. These modifications may enhance the nutritional quality of milk during early lactation. Supplementation may also support milk production by improving energy balance and nutrient partitioning—especially under conditions of high metabolic demand—without negatively affecting DMI [12].

Favorable effects of alpha-linolenic acid (ALA, C18:3) on reproduction have been reported in many studies [13–17]. Omega-3 fatty acids (ALA, EPA, and DHA) have been shown to be effective in reducing the synthesis of prostaglandin F2α in the uterus, facilitating embryo implantation and reducing embryonic mortality, with long-chain fatty acids (EPA and DHA) being the most effective. Alpha-linolenic acid (ALA) can undergo enzymatic desaturation and elongation to form biologically more active compounds such as eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). However, the conversion efficiency in ruminants is limited [18]. Therefore, to enhance the effectiveness of an omega-3-enriched diet, it is necessary to supplement with preformed EPA and DHA to increase their concentrations in plasma and uterine tissues.

Several experiments have evaluated the effectiveness of flaxseed oil as a source of ALA [19,20], as well as fish oil, which provides EPA and DHA [15,16,21], in dairy cow diets. However, the combination of all three fatty acids (ALA, EPA, and DHA) in a single product has not yet been evaluated [3,22]. Based on all the above, the hypothesis of this study was that supplementation with omega-3 PUFAs—including ALA, EPA, and DHA—would improve the metabolic environment, productive performance, and reproductive outcomes. Therefore, to test this hypothesis, we aimed to evaluate the effects of supplementing dairy cows with calcium salts of flaxseed oil enriched with EPA and DHA on productive, metabolic, and reproductive parameters in dairy cows during the first 15 weeks postpartum.

2. Materials and Methods

The experiment was conducted 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). Procedures for animal handling and care were approved (4 August 2020) by the Ethics and Security Committee of the College of Veterinary Sciences—National University of Litoral (Protocol N° 614/20) and have been carried out according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching [23].

2.1. Study Design, Animals, and Treatments

Thirty-six Holstein cows (2.89 ± 1.54 lactations) were distributed to 18 blocks according to probable date of parturition (03/17/2022 ± 16 days), number of lactations (3.32 ± 1.38), body weight (BW; 708 ± 99 kg), body condition score (BCS; 3.34 ± 0.37) and milk yield in the previous lactation (8392 ± 1542 kg of milk), and were randomly assigned within each block to two treatments: Omega-3 (O-3) and Control (C). All values are presented as mean ± standard deviation (SD). The study began 21 ± 2 days prior to the estimated calving date and ended at 105 ± 5 days in milk. All animals were housed together in a single shaded dry-lot pen with covered feeding alleys and ad libitum water availability.

All cows were equipped with a neck transponder for automatic recording of daily milk production on an individual basis (DelPro Farm Manager 6.60/DeLaval, Tumba, Sweden).

During the prepartum period, both treatment groups were offered a mixed partial ration (PMR) fed ad libitum offered in the morning (63.2% corn silage, 12.9% wheat straw, 16.9% soybean meal, 3.8% ground corn, and 3.2% anionic salts, based on dry matter (DM)). The O-3 group received 0.40 kg DM d^{-1} of omega-3 calcium salts and cows in the C group received 0.63 kg DM d^{-1} of ground corn as an isocaloric replacement. This period was used as an acclimation to the lactating cow diets. During the first 15 weeks of lactation, the animals received a PMR ad libitum once a day, after the morning milking (36.5% alfalfa silage, 24.1% corn silage, 18.6% soybean meal, 9.9% ground corn, 7.8% alfalfa hay, 2.1% cottonseed whole, and 0.9% premix vitamins and minerals, based on DM) while in the milking parlor, they received differential supplementation depending on the treatment. The O-3 group supplement contained 4.48 kg DM d^{-1} of pelleted concentrate plus 0.65 kg DM d^{-1} of calcium salts of omega-3, distributed individually in equal parts in each milking shift. The C group supplement was similar to that offered to the O-3 group, but the calcium salts were replaced by isocaloric ground corn grain (1.15 kg DM d^{-1}). To calculate the energy concentration of lipids, the following equation was used: Digestible energy = 9.4 × FA digestibility × EE/100 [24].

The calcium salts (99.4% DM, 69.7% EE, 25.9% ash) had linseed oil and fish oil (60:40), containing 33.5% of omega-3 on the total fatty acids. Before the start of the trial, the degree of protection of the fat supplement was determined, for which the impact of adding it on ruminal fermentation was evaluated through an in vitro digestion system.

The ingredients and nutrients of the experimental diets are presented in Table 1.

Table 1. Dietary ingredients and nutrient composition of diets.

	Treatments [1]	
INGREDIENTS, kg of DM	**O-3**	**C**
Alfalfa hay	1.70	1.70
Corn silage	5.24	5.24
Alfalfa silage	7.94	7.94
Soybean meal	4.04	4.04
Cottonseed	0.46	0.46
Ground corn	2.14	2.14
Mineral/vitamin premix [2]	0.20	0.20
Pellets concentrate [3]	4.48	4.48
Calcium salts O-3	0.65	-
Corn ground (isocaloric replacement)	-	1.15
CHEMICAL COMPOSITION OF DIETS [4]		
Dry Matter, %	53.4	53.7
NEL, Mcal kg	1.71	1.71
Fatty Acids, % DM	4.99	3.40
CP, % DM	18.30	18.40
RDP, % DM	10.80	10.80
RUP, Base, % DM	7.60	7.60
ADF, % DM	19.1	18.90
NDF, % DM	30.0	30.10
Starch, % DM	20.5	23.30
Ash, % DM	9.30	8.60

Abbreviations: NEL, net energy for lactation; CP, crude protein; RDP, rumen degradable protein; RUP, rumen undegradable protein; ADF, acid detergent fiber; NDF, neutral detergent fiber. [1] O-3 = calcium salts of Omega-3; C = Control diet. [2] Calcium, 212.8 g kg^{-1}; sodium, 97.5 g kg^{-1}; chlorine, 145 g kg^{-1}; magnesium, 67.5 g kg^{-1}; copper, 946 mg kg^{-1}; zin, 4482 mg kg^{-1}; manganese, 4482 mg kg^{-1}; iron, 100 mg kg^{-1}; iodine, 75 mg kg^{-1}; selenium, 9.96 mg kg^{-1}; cobalt, 9.96 mg kg^{-1}; sodium monensin, 1300 mg kg^{-1}; vitamin A, 239,044 IU kg^{-1}; vitamin D3, 119,522 IU kg^{-1}; vitamin E, 2400 IU kg^{-1}. [3] Pellets concentrate (DM basis) = 89.6 ± 2.5% DM, 17.3 ± 1.3% CP, 41.7 ± 4.6% starch, 13.3 ± 2.1% NDF, 4.7 ± 0.7% ADF, 5.1 ± 0.8% EE, 9.4 ± 0.5% ash, and 82.5 ± 3.4% in vitro DM digestibility. [4] Predicted with NASEM dairy-8 software (8th ed.) [25] using feed composition.

2.2. Feed Analysis

Representative samples of the concentrate, the PMR, and the ingredients of the ration were collected on a weekly basis. These samples were oven-dried at 65 °C using a forced-air system until a constant weight was reached to determine DM content and then they were ground in a Wiley mill (1 mm mesh). Analytical procedures were carried out to determine ash content [26], crude protein (CP; following Kjeldahl method [27]), neutral detergent fiber (NDF; using ANKOM Technology Method 6-2011 validated with NFTA protocol), acid detergent fiber (ADF; ANKOM Technology Method 5-2011 validated by AOAC 1990, [28]), ether extract (EE; using an automated adaptation of the Soxhlet method [29]), and in vitro DM digestibility (IVDMD; using the two-stage fermentation process described by Tilley and Terry [30]).

2.3. Measurements of Milk and Milk Components

Cows were milked twice daily, and individual milk yields were recorded each day using the DelPro automated measurement system (DelPro Farm Manager 6.60, DeLaval, Tumba, Sweden). Weekly averages were used for analysis. Milk produced during the first week postpartum was classified as transition milk and was excluded from the dataset (designated as week 0). Milk composition was assessed biweekly using individual samples. For each sampling, milk was collected from two consecutive milking sessions (a.m. and p.m.) with DeLaval milk meters, and a composite sample for each cow was prepared by pooling the subsamples proportionally to their respective volumes.

Each pooled sample was analyzed for fat, protein, lactose, total solids, non-fat solids, and urea concentrations using infrared spectrophotometry (MilkoScanTM Minor; FOSS Electric, Hilleroed, Denmark) according to the standard method [31]. Fat-corrected milk (4% FCM) was calculated using the formula described by Gaines & Davidson [32], and energy-corrected milk (ECM) was calculated following the equation proposed by Tyrrell & Reid [33].

Additionally, 100 mL milk samples were collected from each cow during the 5th week of lactation and stored at −24 °C for the FA profile. Fatty acids were converted to fatty acid methyl esters (FAMEs) by transesterification with a methanolic potassium hydroxide solution as an intermediate step prior to saponification [34]. The FAMEs were then analyzed using gas chromatography (GC-2014, Shimadzu) equipped with an automatic injector (AOC-20i), as described in [35].

During the 5th week of lactation, individual 100 mL milk samples were collected from each cow and stored at −24 °C for subsequent fatty acid analysis. The fatty acids were derivatized into methyl esters (FAMEs) through transesterification using a methanolic potassium hydroxide solution, which preceded the saponification step [34]. These FAMEs were then quantified by gas chromatography (GC-2014) equipped with an automatic injector (AOC-20i), both from Shimadzu Corporation, Kyoto, Japan, following the methodology detailed in [35].

2.4. Dry Matter Intake

Individual PMR intake was assessed during the 5th and 15th weeks of the trial by calculating the difference between the amount offered and refused, with cows temporarily housed in individual pens for accurate measurement. Daily concentrate intake was similarly determined by subtracting refusals from the amount offered each day throughout the experimental period. Total DM intake was calculated by summing the DM intake from both the PMR and concentrate.

Total energy intake was calculated from the DMI of PMR and concentrate and their NEL content estimated according to NASEM [25].

2.5. Body Weight and Body Condition Score

Individual body weight (BW) measurements were obtained for all cows using an electronic scale on day –21 relative to calving and on days 7, 21, 35, 60, 75, and 112 postpartum, immediately after the morning milking. Body condition score (BCS) was evaluated at the same time as body weight measurements by two independent evaluators using a 5-point scale, with increments of 0.25 points as described by Wildman et al. [36]. The average score from both evaluators was used for subsequent analysis.

2.6. Blood Sampling, Hormones, and Metabolite Analysis

Blood samples (15 mL) were collected from the coccygeal vein of each cow at the same time as BW measurements. Samples were drawn into tubes containing sodium heparin (5 IU mL^{-1}). Plasma was separated by centrifugation at 2000× g for 15 min at 4 °C and stored in an ultrafreezer (−80 °C) until analysis. Glucose (Enzymatic Glycemia), urea (Uremia), and total cholesterol (Enzymatic Colestat) concentrations were determined using commercial kits (Wiener Laboratory, Rosario, Argentina). Non-esterified fatty acids (NEFA; FA115, Randox Laboratories Ltd., Crumlin, UK), insulin-like growth factor I (IGF-I; measured by radioimmunoassay following acid-ethanol extraction; UB2-495, Hormone Distribution Program, National Institute of Diabetes and Digestive and Kidney Diseases, Rockville, MD, USA), and insulin (measured by radioimmunoassay using anti-bovine insulin antibody, Sigma, St. Louis, MO, USA, and standard human insulin provided by Laboratories Beta, Buenos Aires, Argentina) were analyzed on days –21, 7, 21, and 35. On the same days, beta-hydroxybutyrate (BHBA) was measured in whole blood using the FreeStyle Optium ketone test system (Abbott Diabetes Care Ltd., Witney, UK). All procedures were performed in accordance with the methodologies previously described in [37].

2.7. Reproductive Management

All cows were examined after parturition once a week to make sure they were in optimal condition to receive service at the end of the voluntary waiting period. At 43 ± 5 days postpartum, cows were synchronized with the Presynch–Ovsynch (PSOv) protocol for fixed timed artificial insemination (FTAI) to eliminate variations associated with estrus detection and facilitate management [14]. The day of FTAI was declared as day 0. The PSOv protocol entails (D-36 = PGF2α, D-22 = PGF2α, D-10 = GnRH, D-3 = PGF2α, D-2 = PGF2α, D-1 = GnRH, and 16 h later; D0 = FTAI). Cows in the PSOv treatment received FTAI at 78 ± 5 DIM. For synchronization of ovulation protocols, GnRH treatments consisted of 21 mg of Buserelin acetate given i.m. (Gestar, Over, Santa Fe, Argentina), whereas PGF2α treatments consisted of 150 mg of D-Cloprostenol given i.m. (Prostal, Over, Santa Fe, Argentina). Ovarian activity was assessed by transrectal ultrasonography with a portable ultrasound (PROVETSCAN SR-2C, transrectal lineal transductor 6.5–8 MHz, real-time Mod B, BM, Colour, PDI, PW; New VeTec, León, Spain) to determine the number and size of ovarian structures (follicles [FOL] and corpus luteum [CL]). Follicles with a diameter of ≥3–5 mm were counted and for all follicles greater than 5 mm, the diameter was recorded. The size of ovarian structures was calculated by the average of two perpendicular diameters. The cavity of the CL was calculated the same way and subtracted from the total area. The number of follicles was recorded on day −3 of the PSOv protocol. The presence and size of the CL were evaluated on days 14, 22, and 28 after FTAI. Blood samples for progesterone measurements were collected on days 5, 14, 22, and 28 post FTAI by coccygeal venipuncture. The samples were centrifuged (2000× g for 15 min) and the serum obtained was stored at −18 °C until analyzed for chemiluminescence using a Centaur XP analyzer (Siemens Healthineers, Munich, Germany).

Pregnancy diagnosis was performed by transrectal ultrasonography 32 days after FTAI and confirmed by transrectal palpation of uterine contents approximately 60 days later of FTAI. After day 32 post-FTAI, reproductive management consisted of estrus detection (visual) and once-daily insemination. Cows that failed to conceive following insemination were re-inseminated upon detection of estrus through visual observation by farm personnel. Those not re-inseminated at estrus and confirmed to be non-pregnant 32 $\pm$ 3 days after the previous AI service received either a prostaglandin $F_2\alpha$ i.m. (150 mg D-Cloprostenol, Prostal) treatment in the presence of a corpus luteum, or a gonadotropin-releasing hormone i.m. (21 mg of Buserelin acetate, Gestar) followed by prostaglandin (150 mg D-Cloprostenol, Prostal) seven days later in the absence of a corpus luteum. The proportion of pregnant cows up to 130 DIM was evaluated.

2.8. Statistical Analysis

Results for milk production and composition, BW, BCS, DMI, luteal area, and plasma metabolites and hormones were analyzed according to a randomized complete block design with repeated observations in time, adjusted by covariate ($\alpha = 0.05$). The following model was used: $Y_{ijkl} = \mu + T_i + B_j + A\ (B)_{k(j)} + W_l + (T \times W)_{il} + Cov + E_{ijkl}$, where Y_{ijkl} = dependent variable, μ = general mean, T_i = treatment effect, B_j = block effect, A $(B)_{k(j)}$ = random effect of animal nested to block, W_l = sampling week effect, $(T \times W)_{il}$ = effect of treatment interaction $\times$ sampling week, Cov = covariate, and E_{ijkl} = residual error.

The large of preovulatory follicle was analyzed using a standard analysis of variance within a randomized complete block design. The statistical model used was $Y_{ijk} = \mu + T_i + B_j + E_{ijk}$, where Y_{ijk} = represents the dependent variable for cow k in block j under treatment i, μ = is the overall mean, T_i = is the fixed effect of treatment, B_j = is the random effect of block, and Eijk = is the residual error.

Results for cows ovulated for FTAI and the conception of those ovulated were analyzed by means of a model with a classification criterion (treatment): $Y_{ijk} = \mu + T_i + B_j + A\ (B)_{k(j)} + E_{ijk}$, where Y_{ijk} = dependent variable, μ = general mean, T_i = treatment effect, B_j = block effect, A $(B)_{k(j)}$ = random effect of animal nested to block, and Eijk = residual error. All statistical analyses were performed using R software v4.2.2 [38].

To analyze the proportion of cows that were pregnant by 130 DIM, the non-parametric Kaplan–Meier method was applied to estimate the survival function for each treatment group. Differences between the survival curves of the treatment groups were assessed using the log-rank test, with a significance threshold set at $p < 0.05$. The analysis was conducted using R software v4.2.2 [38], following established methodological guidelines to ensure the reproducibility and validity of the results.

All model assumptions were verified by testing the normality of residuals using the Anderson–Darling test (nortest package in R). For all the analyses, explanatory variables and their interactions were considered significant if $p \leq 0.05$ whereas $0.05 < p \leq 0.10$ was considered a tendency.

3. Results

3.1. Milk Production and Composition

A significant treatment $\times$ week interaction was detected for milk yield ($p < 0.01$; Table 2). Milk production was higher in cows supplemented at only 6 out of 14 weeks of lactation analyzed ($p < 0.05$). In addition, during weeks 3, 4, and 7, a trend toward higher milk production was observed in the O-3 group (Figure 1).

Table 2. Milk production and composition in dairy cows supplemented (O-3) or not (C) with PUFA calcium salts (0.65 kg·day^{-1}).

	Treatment [1]		SEM	*p*-Value		
	O-3	C		Treatment	Time	Treatment × Time
Milk, kg d^{-1}	37.0	34.0	1.08	0.01	<0.01	<0.01
4% FCM, kg d^{-1}	35.1	33.3	1.31	0.31	<0.01	0.99
ECM, kg d^{-1}	34.9	32.5	1.24	0.17	<0.01	0.96
Butterfat						
%	3.72	3.68	0.10	0.60	0.09	0.97
kg d^{-1}	1.36	1.28	0.05	0.19	<0.01	0.63
Total Protein						
%	3.19	3.13	0.04	0.18	<0.01	0.79
kg d^{-1}	1.18	1.06	0.04	0.03	<0.01	0.41
Lactose, %	4.97	4.91	0.04	0.35	<0.01	0.05
Total solids, %	12.7	12.7	0.16	0.96	<0.01	0.60
Non-fat solids, %	8.91	8.91	0.09	0.66	<0.01	<0.01
MUN, mg 100 mL^{-1}	35.7	36.5	0.72	0.27	<0.01	0.13

[1] Values are expressed as least squares means (LSMeans) and the standard error of the LSMeans (SEM). Abbreviations: 4% FCM: 4% fat-corrected milk; ECM: energy-corrected milk; MUN: Milk Urea Nitrogen.

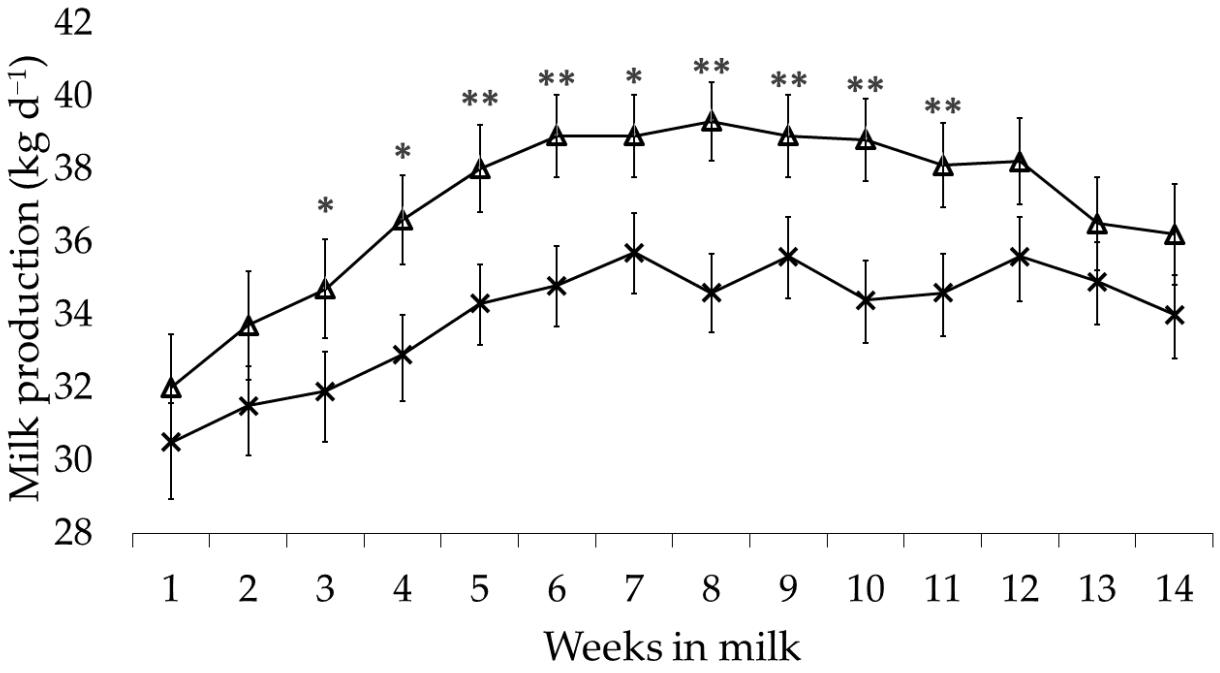

Figure 1. Milk yield in dairy cows supplemented (O-3; —△—) with PUFA omega-3 (0.65 kg·day^{-1}) or not supplemented (C; —×—). Tendencies at * $p < 0.10$, and significances at ** $p < 0.05$. Error bars represent SEM.

Cows in the O-3 group showed a consistent increase in milk production from week 1 (32.0 kg/day), reaching a peak at week 8 (39.3 kg/day). This peak was followed by a period of relatively stable production for approximately four weeks, after which a slight decline was observed toward the end of the study (week 13: 36.5 kg/day; week 14: 36.2 kg/day). In contrast, the Control group reached a lower peak production of 35.7 kg/day at week 7, followed by a gradual decline through week 14 (34.0 kg/day). These patterns support a significant treatment*time interaction, indicating that the O-3 group not only achieved a higher peak but also maintained milk production more persistently over time compared to the C group.

The FCM, ECM, butterfat and solids contents were similar ($p > 0.10$) between treatments, whereas protein yield was significantly higher (11.3% increment, $p < 0.05$) in the cows from the O-3 group (Table 2).

3.2. Dry Matter and Energy Intake

Dry matter intake (DMI) of the concentrate offered in the parlor was greater ($p < 0.01$) for cows in the C group than in the O-3 group (Table 3). This result could be explained by greater ($p < 0.01$) refusals observed in the O-3 group, in addition to the fact that, due to the trial design (isoenergetic concentrates), the cows in the C group received +0.5 kg DM day^{-1} of concentrate. The DM intake of PMR and total DM intake were similar ($p > 0.10$) between treatment groups (Table 3).

Table 3. Dry matter and energy intake in dairy cows supplemented (O3) or not (C) with PUFA calcium salts (0.65 kg·day^{-1}).

	Treatment [1]		SEM	*p*-Value		
	O-3	C		Treatment	Time	Treatment × Time
CONCENTRATE [2]						
Offered, kg DM d^{-1}	5.13	5.63				
Refused, kg DM d^{-1}	0.73	0.33	0.08	<0.01	<0.01	0.20
DM Intake, kg d^{-1}	4.41	5.30	0.08	<0.01	<0.01	0.22
NEL intake [3], Mcal d^{-1}	10.0	11.5	0.22	<0.01	<0.01	0.11
PMR						
DM Intake, kg d^{-1}	22.6	21.9	0.38	0.17	<0.01	0.43
NEL intake [3], Mcal d^{-1}	35.9	35.0	1.13	0.34	<0.01	0.43
TOTAL						
DM Intake, kg d^{-1}	27.0	27.2	0.37	0.70	<0.01	0.59
NEL intake [3], Mcal d^{-1}	45.9	46.6	1.13	0.94	<0.01	0.14
Energy balance [4], Mcal d^{-1}	6.15	7.89	0.69	0.06	<0.01	0.61
ECM/DM intake	1.28	1.20	0.03	0.03	0.89	0.71
ECM/NEL intake	0.77	0.72	0.02	0.05	0.18	0.74

[1] Values are expressed as least squares means (LSMeans) and the standard error of the LSMeans (SEM). [2] O-3: pellets concentrate + calcium salts O-3; C: pellets concentrate + ground corn (isocaloric replacement). [3] Estimated according to [25]. [4] Estimated according to [25] using DM intake data obtained during the 5th and 15th week of the experimental period.

Dry matter intake and energy intake from PMR as well as the total (concentrate + PMR) were similar between groups (Table 3). A tendency for a lower energy balance was observed in the O-3 group relative to the Control group (Table 3). Feed efficiency was different between groups, whereby cows receiving by-pass fat produced significantly more ECM per kilogram of DM intake and per Mcal of NEL intake (Table 3).

3.3. BW, BCS, Metabolites and Hormones

No treatment effect was detected for body weight (BW) or body condition score (BCS). Plasma NEFA concentrations tended to be greater ($p < 0.10$) in cows from the O-3 group. In lipid-supplemented cows, BHBA concentrations were greater than in the C group. Plasma concentrations of glucose, insulin, and urea were similar between treatments ($p > 0.10$), whereas circulating cholesterol levels were significantly greater (by 19.3%; $p < 0.05$) in cows from the O-3 group (Table 4).

Table 4. BW, BCS, metabolites, and hormones in dairy cows supplemented (O3) or not (C) with PUFA calcium salts (0.65 kg·day^{-1}).

	Treatment [1]		SEM	*p*-Value		
	O-3	C		Treatment	Time	Treatment × Time
BW, kg	658	646	6.68	0.17	<0.01	0.66
BCS	3.31	3.25	0.05	0.32	<0.01	0.61
NEFA, mmol L−^{1}	0.62	0.55	0.03	0.07	<0.01	0.70
BHBA, mmol L^{-1}	0.97	0.82	0.08	0.05	<0.01	0.73
Insulin, ng mL^{-1}	0.42	0.57	0.10	0.21	0.12	0.11
IGF-1, ng mL^{-1}	135	136	9.77	0.94	0.68	0.07
Glucose, g L^{-1}	0.59	0.60	0.01	0.39	<0.01	0.50
Urea, g L^{-1}	0.43	0.42	0.01	0.33	<0.01	0.94
Cholesterol, mg dL^{-1}	182	160	6.77	0.01	<0.01	0.13

[1] Values are expressed as least squares means (LSMeans) and the standard error of the LSMeans (SEM). BW = body weight; BCS = body condition score.

A tendency for a treatment-by-time interaction was detected for IGF-1; however, within each day, no significant differences were observed between groups (Table 4).

3.4. Ovarian Structures and Reproductive Performance

A tendency was observed in the proportion of cows that ovulated at the time of FTAI ($p < 0.10$), with 17 out of 18 cows (94.4%) ovulating in the O-3 group compared to 13 out of 18 cows (72.2%) in the Control group. No differences were found ($p > 0.10$) in the number of follicles per ovary and the size of the preovulatory follicle (Table 5).

Table 5. Reproductive parameters in dairy cows supplemented (O3) or not (C) with PUFA calcium salts (0.65 kg·day $^{-1}$).

	Treatment [1]		SEM	*p*-Value
	O-3	C		
Ovulated to FTAI (%)	94.44	72.22	0.12	0.08
Number of follicles	3.27	2.54	0.32	0.11
Size of preovulatory follicle (mm)	16.18	16.21	0.99	0.97
Pregnancies per AI (%)	55.55	38.88	0.09	0.33
Proportion of cow pregnant by 130 DIM (%)	76.47	56.25	0.12	0.02

[1] Values are expressed as least squares means (LSMeans) and the standard error of the LSMeans (SEM).

Pregnancies per AI (P/AI) at first insemination in cows was not affected ($p > 0.10$) by the treatments (Table 5). However, differences ($p < 0.05$) were observed in favor of the supplemented cows regarding pregnancy progression at 130 days postpartum. Only one case of early pregnancy loss (between 32 and 60 days after FTAI) was recorded in the O-3 group, while none occurred in the C group.

Serum progesterone concentrations showed a significant treatment * time interaction ($p < 0.05$; Figure 2a). Although no differences were detected on days 5 and 22, cows in the O-3 group tended to have higher concentrations on day 14 ($p < 0.10$), and exhibited significantly higher levels on day 28 compared to the control group ($p < 0.01$). No significant differences were observed in luteal area measurements on days 14, 22, or 28 post-FTAI ($p > 0.10$; Figure 2b).

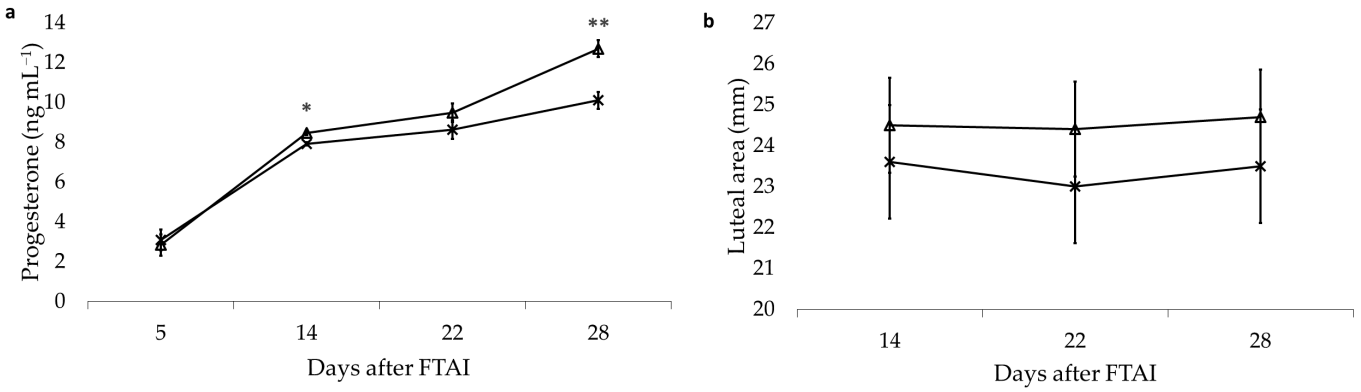

Figure 2. Progesterone concentration (**a**) and luteal area (**b**) in dairy cows supplemented (O-3; —△—) with PUFA omega-3 (0.65 kg·day^{-1}) or not supplemented (C; —×—). Tendencies at * $p < 0.10$, and significances at ** $p < 0.05$. Error bars represent SEM.

4. Discussion

During the TP, the ability to ingest energy through voluntary intake is typically lower than the energy required for colostrum and milk synthesis at the onset of lactation, leading to a negative energy balance (NEB) that may begin before calving and extend for several weeks into lactation [39]. The magnitude of NEB depends on various factors, such as voluntary intake, milk production, and body condition. In the present study, supplementation with omega-3 PUFA in the diet of early-lactation dairy cows improved milk production and feed efficiency without affecting total DMI, NEL intake, BW, or BC.

Although previous studies have reported a negative effect of lipid supplementation on DMI [8,40], the findings are inconsistent. This variability is likely due to differences in the physicochemical characteristics of the supplement, the types of fatty acid supplemented (e.g., chain length, degree of saturation, and source), the level of dietary inclusion, and the stage of lactation [40]. In their review, Block and Evans [41] reported a reduction in DMI when dietary supplementation of omega-3 PUFA exceeded 60 g kg^{-1} of DM (6% of average DMI). In the present study, the level of supplementation was 2.4% of the average DMI, which may help explain the similar total DMI observed across treatments. Moallem [40] also noted that DMI may be negatively affected when flax oil inclusion exceeds 3% and fish oil exceeds 1% of dietary DM. In our study, flax and fish oil inclusion levels were approximately 1.44% and 0.96% of dietary DM, respectively.

Nonetheless, concentrate DM intake was higher in cows from the C group. This outcome may be attributed to greater concentrate refusal observed in the O-3 group, as well as the trial design, which included isoenergetic concentrates, resulting in cows from the C group receiving an additional 0.5 kg DM d^{-1} of concentrate. In dairies where concentrate is offered for a limited time in the milking parlor, as in this and other studies we have conducted [42], a lower intake rate and meal size have been associated with decreases in concentrate DMI when supplemental fats are included in the ration [43], highlighting an important consideration for ration formulation and feeding management. Consequently, the estimated total NEL intake was similar between treatment groups. This finding aligns with a previous report that found no effect on NEL intake with the inclusion of 0.6 kg d^{-1} of calcium salts of fatty acids [44]. Therefore, in the present study, no evidence of a negative impact of lipid supplementation on total DMI or NEL intake was observed.

Lipid supplementation increased both milk and milk protein production (kg d^{-1}). Similarly, Petit et al. [45] reported higher milk production during early lactation in cows supplemented with omega-3 PUFA compared to those fed a lipid-free control diet. In the present study, cows in the O-3 treatment group also demonstrated greater efficiency in converting dry matter and energy intake into milk (ECM/DMI and ECM/NEL intake). This improvement in efficiency is consistent with previous findings showing enhanced feed-to-milk conversion when rations are supplemented with rumen-protected fats [42]. Two primary mechanisms may explain this response. First, bypass lipids can reduce energy losses in the form of methane compared to carbohydrates, due to the decreased availability of fermentable carbohydrates in the rumen when lipids isoenergetically replace corn grain [46]. Second, the net effect of protected lipid supplementation on energy utilization efficiency by the mammary gland may be attributed to the balance between increased uptake of dietary fatty acids and reduced de novo fatty acid synthesis [43]. The direct uptake of long-chain fatty acids by the mammary gland lowers the energy cost associated with fatty acid synthesis for milk fat secretion [44,47]. In this study, the concentration of preformed FA (>C16:0) in milk fat was higher in lipid-supplemented cows (46.7 vs. 41.3 g 100 g^{-1} FA for the O-3 and C groups, respectively), while the concentration of de novo synthesized fatty acids (<C16:0) was lower (24.3 vs. 26.3 g 100 g^{-1} FA, respectively). This shift in milk fatty acid composition may partially explain the greater energy utilization efficiency observed in the O-3 group. These findings are consistent with those of Sun et al., who reported that omega-3 supplementation in lactating cows reduced the proportion of de novo synthesized fatty acids and increased the proportion of long-chain fatty acids (LCFAs, >C16:0) in milk fat [48]. Additionally, omega-3 PUFAs are modulators of the expression of Peroxisome Proliferator-Activated Receptors (PPARs), which could influence nutrient utilization efficiency, improving insulin sensitivity [49]. Therefore, they are proposed as key molecules regulating energy homeostasis in dairy cows [50], which could help explain the greater efficiency in ECM production per kg of DMI and NEL intake observed in cows from the O-3 group.

In response to NEB, the body increases lipomobilization [51], resulting in BW loss, reduced BCS, and elevated concentrations of NEFA and BHBA [52]. In this study, circulating NEFA and BHBA levels tended to increase in cows from the O-3 group. Coincidentally, a trend towards a lower energy balance was observed in the O-3 group relative to the C group. However, these differences were not reflected in BW or BCS. This supports previous findings suggesting that BW and BCS may not be sensitive or reliable indicators of lipomobilization in transition cows [52]. According to Duffield et al., subclinical ketosis is characterized by BHB concentrations > 1.2 mmol L^{-1}, NEFA > 0.7 mmol L^{-1}, and hypoglycemia (glucose < 0.36 g L^{-1}) [53]. In this study, average circulating concentrations of NEFA and BHB remained below these thresholds, and glucose levels stayed above the hypoglycemia cutoff in both treatment groups throughout the TP. Fat supplementation has shown inconsistent effects on circulating glucose and insulin concentrations [54]. However, when fat replaces starch in the diet, a reduction in propionate supply may occur, potentially limiting hepatic gluconeogenesis and insulin secretion. Despite this, plasma glucose and insulin concentrations did not differ between treatments in the present study. This stability may be partially attributed to the absorption of fatty acids from rumen-protected lipids, which can help maintain glycemia by decreasing total glucose oxidation (via CO_2 production) or partial oxidation to $NADPH_2$ [55].

Lipid supplementation has been shown to increase circulating cholesterol levels [20,56]. In our study, supplementation with calcium salts of PUFA led to elevated plasma cholesterol concentration, a finding that aligns with the higher circulating progesterone levels observed in the O-3 group. Cholesterol is the primary substrate for progesterone synthesis in the corpus luteum [57], which enhances the proliferation, survival, and viability of embryonic cells [58,59]. Although, Elis et al. suggested that the inclusion of omega-3 PUFA in the diet may improve ovarian function by increasing the size and estradiol production in the preovulatory follicle [16]. However, in this study, no differences were observed in the number and size of the follicles, nor in the size of the corpus luteum, between treatment groups in this study. Nonetheless, the higher progesterone concentration in cows from the O-3 group may explain the significantly greater proportion of cows who were pregnant at 130 DIM, 76.5% compared to 56.3% in the C group, a difference of 20.2 percentage points. In line with this, a numerical increase was observed in both the ovulation and pregnancy rates in the O-3 group; however, these differences did not reach statistical significance. This is likely due to the limited number of animals per group (n = 18), which reduced the statistical power to detect differences, particularly in pregnancy outcomes. Given that ovulation is a prerequisite for pregnancy, and considering that five cows in the control group failed to ovulate, ovulation rate represents a more robust and biologically relevant indicator in this context. Therefore, results related to pregnancy should be interpreted with caution, as the sample size may not be sufficient to draw definitive conclusions regarding treatment effects on fertility.

PUFA supplementation has been shown to have positive effects on reproduction when supplemented both during the prepartum and the breeding periods [16,60,61]. Jolazadeh et al. reported improved conception rates and reduced days open in cows fed calcium salts (1.5% of DM) of fish oil rich in EPA and DHA from 21 days before calving until parturition [21]. Similarly, Moallem et al. found that feeding dairy cows 2.5% of DM of fish oil from 14 days prepartum through 100 DIM increased the number of follicles suitable for ovum pickup [15]. Changes in the fatty acid profile of the diet affect the fatty acid composition of the follicular fluid [62] and the lipid composition of the follicular fluid, which promotes oocyte maturation and embryonic development [63]. Moallem et al. also showed that cows fed encapsulated flaxseed oil had a 2.5-fold higher concentration of alpha-linolenic acid (ALA) in their follicular fluid compared to cows fed encapsulated saturated fats [15]. This suggests that the dietary fatty acid profile could play a key role in the lifespan of the corpus luteum by acting through intrinsic ovarian factors. Additionally, supplementation with O-3 blocks the synthesis of bovine endometrial PGF2α in vitro and in vivo, resulting in improved fertility and embryonic survival [64].

5. Conclusions

Under the conditions of the present study, dietary supplementation with a blend of linseed oil (source of ALA) and fish oil (source of EPA and DHA) during early lactation increased milk and protein yield without altering milk composition. This increase occurred without a corresponding rise in energy intake, the improved production may be attributed to enhanced energy utilization efficiency. The results also suggest that the energy provided in the form of lipids during early lactation would not predispose cows to improve body condition.

Lipid supplementation elevated plasma cholesterol and progesterone concentrations and improved the proportion of cows who were pregnant by 130 DIM. These findings indicate that this supplementation strategy holds promise for enhancing both productivity and reproductive performance. However, further studies with larger sample sizes are necessary to confirm the reproductive benefits.

Author Contributions: Investigation, methodology, data curation, formal analysis, visualization, writing—original draft, P.M.R.; data curation, formal analysis, A.C.; review and editing, M.S.; methodology, G.S.; methodology, review and editing, E.A.; resources, supervision, project administration, review and editing, G.J.H.; resources, supervision, project administration, review and editing, E.E.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Instituto Nacional de Tecnología Agropecuaria (INTA), through projects PD-L01-I118 and PD-L01-I130, and by the Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación, through project PICT-2021-I-A-00535.

Institutional Review Board Statement: The animal study protocol was approved by the Ethics and Security Committee of the College of Veterinary Sciences, National University of Litoral (protocol code 614/20). The study was conducted 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), and followed the guidelines of the Guide for the Care and Use of Agricultural Animals in Research and Teaching [23].

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study may be available upon reasonable request from the corresponding author. Due to institutional requirements for the author's doctoral thesis, the dataset must remain unpublished until the thesis evaluation process is complete. Following this process, the data could be made publicly available.

Acknowledgments: We thank TECNUAR SRL (Va. Gdor. Gálvez, Santa Fe, Argentina) for the production of calcium salts. Special thanks to the students (Lucas, Mayka, Nadia, Antonella, Micaela, Salvador, and Felipe) and to Martín Maciel, Gabriel Schmidt, and Darío Arias for their assistance during the methodological procedures and sampling.

Conflicts of Interest: The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Abbreviations

ADF	Acid detergent fiber
AI	Artificial insemination
ALA	Alpha-linolenic acid
BCS	Body condition score
BHBA	Beta-hydroxybutyrate
BW	Body weight
C	Control treatment group
CL	Corpus Luteum
CP	Crude protein
DHA	Docosahexaenoic acid
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
ECM	Energy-corrected milk
EPA	Eicosapentaenoic acid
FA	Fatty acid
FCM	Fat-corrected milk
FOL	Follicle
FTAI	Fixed-Time Artificial Insemination
GnRH	Gonadotropin-Releasing Hormone
IGF-I	Insulin-like growth factor I
NDF	Neutral detergent fiber
NEB	Negative energy balance

NEFA	Non-esterified fatty acids
NEL	Net energy for lactation
O-3	Omega-3 treatment group
$PGF_2\alpha$	Prostaglandin F2 alpha
PSOv	Presynch–Ovsynch
PUFA	Polyunsaturated fatty acid
RDP	Rumen degradable protein
PMR	Mixed partial ration
RUP	Rumen undegradable protein
TP	Transition period

References

1. LeBlanc, S.J.; Osawa, T.; Dubuc, J. Reproductive Tract Defense and Disease in Postpartum Dairy Cows. *Theriogenology* **2011**, *76*, 1610–1618. [CrossRef] [PubMed]
2. Angeli, E.; Rodríguez, F.M.; Rey, F.; Santiago, G.; Matiller, V.; Ortega, H.H.; Hein, G.J. Liver Fatty Acid Metabolism Associations with Reproductive Performance of Dairy Cattle. *Anim. Reprod. Sci.* **2019**, *208*, 106104. [CrossRef] [PubMed]
3. Carneiro, L.C.; Williams, E.J.; Saut, J.P.E.; Dos Santos, R.M.; Celeghini, E.C.C. The Effect of N-3 Polyunsaturated Fatty Acid Supplementation Oimmune and Reproductive Parameters in Dairy Cows: A Review. *Braz. J. Vet. Res. Anim. Sci.* **2021**, *58*, e175224. [CrossRef]
4. Santos, J.E.P.; Bilby, T.R.; Thatcher, W.W.; Staples, C.R.; Silvestre, F.T. Long Chain Fatty Acids of Diet as Factors Influencing Reproduction in Cattle. *Reprod. Domest. Anim.* **2008**, *43*, 23–30. [CrossRef]
5. Soydan, E.; Şen, U.; Şirin, E. Relationship Between Dietary Fatty Acids and Reproductive Functions in Dairy Cattle. *Turkish J. Agric. Sci. Technol.* **2017**, *5*, 1575–1579. [CrossRef]
6. Sampath, H.; Ntambi, J.M. Polyunsaturated Fatty Acid Regulation of Genes of Lipid Metabolism. *Annu. Rev. Nutr.* **2005**, *25*, 317–340. [CrossRef]
7. Bionaz, M.; Vargas-Bello-Pérez, E.; Busato, S. Advances in Fatty Acids Nutrition in Dairy Cows: From Gut to Cells and Effects on Performance. *J. Anim. Sci. Biotechnol.* **2020**, *11*, 110. [CrossRef]
8. Rabiee, A.R.; Breinhild, K.; Scott, W.; Golder, H.M.; Block, E.; Lean, I.J. Effect of Fat Additions to Diets of Dairy Cattle on Milk Production and Components: A Meta-Analysis and Meta-Regression. *J. Dairy Sci.* **2012**, *95*, 3225–3247. [CrossRef] [PubMed]
9. Bernal-Santos, G.; Perfield, J.W.; Barbano, D.M.; Bauman, D.E.; Overton, T.R. Production Responses of Dairy Cows to Dietary Supplementation with Conjugated Linoleic Acid (CLA) During the Transition Period and Early Lactation. *J. Dairy Sci.* **2003**, *86*, 3218–3228. [CrossRef]
10. Glasser, F.; Ferlay, A.; Doreau, M.; Schmidely, P.; Sauvant, D.; Chilliard, Y. Long-Chain Fatty Acid Metabolism in Dairy Cows: A Meta-Analysis of Milk Fatty Acid Yield in Relation to Duodenal Flows and De Novo Synthesis. *J. Dairy Sci.* **2008**, *91*, 2771–2785. [CrossRef]
11. Moreno González, Y.; Iorio, J.; Olmeda, F.M.; Curletto, D.; Scandolo, D.; Maciel, M.G.; Cuatrin, A.; Palladino, A.R.; Pérez, C.; Salado, E.E. Feeding Calcium Salts of Linseed Oil on Productive Performance and Milk Fatty Acid Profile in Grazing Dairy Cows. *Open J. Anim. Sci.* **2020**, *10*, 761–781. [CrossRef]
12. Onetti, S.G.; Grummer, R.R. Response of Lactating Cows to Three Supplemental Fat Sources as Affected by Forage in the Diet and Stage of Lactation: A Meta-Analysis of Literature. *Anim. Feed Sci. Technol.* **2004**, *115*, 65–82. [CrossRef]
13. Petit, H.V.; Dewhurst, R.J.; Scollan, N.D.; Proulx, J.G.; Khalid, M.; Haresign, W.; Twagiramungu, H.; Mann, G.E. Milk Production and Composition, Ovarian Function, and Prostaglandin Secretion of Dairy Cows Fed Omega-3 Fats. *J. Dairy Sci.* **2002**, *85*, 889–899. [CrossRef]
14. Ambrose, D.J.; Kastelic, J.P.; Corbett, R.; Pitney, P.A.; Petit, H.V.; Small, J.A.; Zalkovic, P. Lower Pregnancy Losses in Lactating Dairy Cows Fed a Diet Enriched in α-Linolenic Acid. *J. Dairy Sci.* **2006**, *89*, 3066–3074. [CrossRef] [PubMed]
15. Moallem, U.; Shafran, A.; Zachut, M.; Dekel, I.; Portnick, Y.; Arieli, A. Dietary α-Linolenic Acid from Flaxseed Oil Improved Folliculogenesis and IVF Performance in Dairy Cows, Similar to Eicosapentaenoic and Docosahexaenoic Acids from Fish Oil. *Reproduction* **2013**, *146*, 603–614. [CrossRef]
16. Elis, S.; Freret, S.; Desmarchais, A.; Maillard, V.; Cognié, J.; Briant, E.; Touzé, J.-L.; Dupont, M.; Faverdin, P.; Chajès, V.; et al. Effect of a Long Chain N-3 PUFA-Enriched Diet on Production and Reproduction Variables in Holstein Dairy Cows. *Anim. Reprod. Sci.* **2016**, *164*, 121–132. [CrossRef] [PubMed]

17. Iorio, J.D.; Gonzalez, Y.M.; Olmeda, M.F.; Curletto, D.; Scandolo, D.E.; Maciel, M.G.; Palladino, R.A.; Giuliodori, M.J.; Salado, E.E. Feeding Calcium Salts of Linseed Oil on Metabolic Status and Reproductive Traits in Grazing Dairy Cows. *Open J. Anim. Sci.* **2024**, *14*, 219–233. [CrossRef]
18. Burdge, G.C.; Calder, P.C. Conversion of Alpha-Linolenic Acid to Longer-Chain Polyunsaturated Fatty Acids in Human Adults. *Reprod. Nutr. Dev.* **2005**, *45*, 581–597. [CrossRef]
19. Dirandeh, E.; Towhidi, A.; Zeinoaldini, S.; Ganjkhanlou, M.; Ansari Pirsaraei, Z.; Fouladi-Nashta, A. Effects of Different Polyunsaturated Fatty Acid Supplementations during the Postpartum Periods of Early Lactating Dairy Cows on Milk Yield, Metabolic Responses, and Reproductive Performances1. *J. Anim. Sci.* **2013**, *91*, 713–721. [CrossRef]
20. Castro, T.; Martinez, D.; Isabel, B.; Cabezas, A.; Jimeno, V. Vegetable Oils Rich in Polyunsaturated Fatty Acids Supplementation of Dairy Cows' Diets: Effects on Productive and Reproductive Performance. *Animals* **2019**, *9*, 205. [CrossRef] [PubMed]
21. Jolazadeh, A.R.; Mohammadabadi, T.; Dehghan-banadaky, M.; Chaji, M.; Garcia, M. Effect of Supplementing Calcium Salts of N-3 and n-6 Fatty Acid to Pregnant Nonlactating Cows on Colostrum Composition, Milk Yield, and Reproductive Performance of Dairy Cows. *Anim. Feed Sci. Technol.* **2019**, *247*, 127–140. [CrossRef]
22. Angeli, E.; Barcarolo, D.; Ribas, L.; Marelli, B.E.; Roskopf, P.M.; Salado, E.E.; Ortega, H.H.; Hein, G.J. Relevant Aspects of Dietary N-3 Polyunsaturated Fatty Acids in the Adaptation of Dairy Cattle to the Transition Period. *Livest. Sci.* **2020**, *244*, 104346. [CrossRef]
23. McGlone, J.; Swanson, J.; Ford, S.; Mitloehner, F.; Grandin, T.; Ruegg, P.; Stull, C.; Lewis, G.; Underwood, W.; Mench, J. *Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 3rd ed.; Federation of Animal Science Societies (FASS): Champaign, IL, USA, 2010; ISBN 9781884706110.
24. National Research Council 2001 (NRC). *Nutrient Requirements of Dairy Cattle*, 7th ed.; National Academies Science: Washington, DC, USA, 2001.
25. National Academies of Sciences, Engineering, and Medicine. *NASEM—Nutrient Requirements of Dairy Cattle*, 8th ed.; National Academies Press: Washington, DC, USA, 2021; ISBN 978-0-309-67777-6.
26. Association of Official Analytical Chemists. AOAC 942.05. In *Official Methods of Analysis*, 15th ed.; AOAC: Frederick, MD, USA, 1990.
27. Association of Official Analytical Chemists. AOAC 976.05. In *Official Methods of Analysis*, 15th ed.; AOAC: Frederick, MD, USA, 1990.
28. Association of Official Analytical Chemists. AOAC 973.18. In *Official Methods of Analysis*, 15th ed.; AOAC: Frederick, MD, USA, 1990.
29. Association of Official Analytical Chemists. AOAC 920.39. In *Official Methods of Analysis*, 16th ed.; AOAC: Frederick, MD, USA, 1998.
30. Tilley, J.M.A.; Terry, R.A. A Two-Stage Technique for in Vitro Digestion of Forage Crops. *Grass Forage Sci.* **1963**, *18*, 104–111. [CrossRef]
31. *ISO 9622/IDF 141*; Milk and Liquid Milk Products—Guidelines for the Application of Mid-Infrared Spectrometry. ISO: Geneva, Switzerland, 2013.
32. Gaines, W.L.; Davidson, F.A. *Relation between Percentage Fat Content and Yield of Milk: Correction of Milk Yield for Fat Content*; University of Illinois Agricultural Experiment Station: Champaign, IL, USA, 1923; pp. 275–620.
33. Tyrrell, H.F.; Reid, J.T. Prediction of the Energy Value of Cow's Milk. *J. Dairy Sci.* **1965**, *48*, 1215–1223. [CrossRef] [PubMed]
34. *ISO 5509*; Animal and vegetable fats and oils—Preparation of methyl esters of fatty acids. ISO: Geneva, Switzerland, 2000.
35. Masson, L.; Alfaro, T.; Camilo, C.; Carvalho, A.; Illesca, P.; Torres, R.; Do Carmo, M.T.; Mancini-Filho, J.; Bernal, C. Fatty Acid Composition of Soybean/Sunflower Mix Oil, Fish Oil and Butterfat Applying the AOCS Ce 1j-07 Method with a Modified Temperature Program. *Grasas y Aceites* **2015**, *66*, e064. [CrossRef]
36. Wildman, E.E.; Jones, G.M.; Wagner, P.E.; Boman, R.L.; Troutt, H.F.; Lesch, T.N. A Dairy Cow Body Condition Scoring System and Its Relationship to Selected Production Characteristics. *J. Dairy Sci.* **1982**, *65*, 495–501. [CrossRef]
37. Schroeder, G.F.; Gagliostro, G.A.; Becu-Villalobos, D.; Lacau-Mengido, I. Supplementation with partially hydrogenated oil in grazing dairy cows in early lactation. *J. Dairy Sci.* **2002**, *85*, 580–594. [CrossRef]
38. R Core Team. R: A Language and Environment for Statistical Computing 2022. Available online: https://www.R-project.org/ (accessed on 10 June 2025).
39. Grummer, R.R. Impact of Changes in Organic Nutrient Metabolism on Feeding the Transition Dairy Cow. *J. Anim. Sci.* **1995**, *73*, 2820–2833. [CrossRef]
40. Moallem, U. Invited Review: Roles of Dietary n-3 Fatty Acids in Performance, Milk Fat Composition, and Reproductive and Immune Systems in Dairy Cattle. *J. Dairy Sci.* **2018**, *101*, 8641–8661. [CrossRef]

41. Block, E.; Evans, E. A Model to Compare Effects of Supplemental Fat Sources on Performance and Dry Matter Intake in Dairy Cows: Effects of Fat Inclusion Level. *J. Dairy Sci.* **2010**, *93*, 440.
42. Roskopf, P.M.; Tieri, M.P.; Cuatrin, A.; Ceron Cucchi, M.E.; Gere, J.I.; Salado, E.E. Performance of Dairy Cows Supplemented with By-Pass Fat under Heat Stress Conditions. *Open J. Anim. Sci.* **2023**, *13*, 82–97. [CrossRef]
43. Schroeder, G.F.; Gagliostro, G.A.; Bargo, F.; Delahoy, J.E.; Muller, L.D. Effects of Fat Supplementation on Milk Production and Composition by Dairy Cows on Pasture: A Review. *Livest. Prod. Sci.* **2004**, *86*, 1–18. [CrossRef]
44. dos Santos Neto, J.M.; Silva, J.O.; Meschiatti, M.A.P.; de Souza, J.; Negrão, J.A.; Lock, A.L.; Santos, F.A.P. Increasing Levels of Calcium Salts of Palm Fatty Acids Affect Production Responses during the Immediate Postpartum and Carryover Periods in Dairy Cows. *J. Dairy Sci.* **2022**, *105*, 9652–9665. [CrossRef] [PubMed]
45. Petit, H.V.; Germiquet, C.; Lebel, D. Effect of Feeding Whole, Unprocessed Sunflower Seeds and Flaxseed on Milk Production, Milk Composition, and Prostaglandin Secretion in Dairy Cows. *J. Dairy Sci.* **2004**, *87*, 3889–3898. [CrossRef]
46. Johnson, K.A.; Kincaid, R.L.; Westberg, H.H.; Gaskins, C.T.; Lamb, B.K.; Cronrath, J.D. The Effect of Oilseeds in Diets of Lactating Cows on Milk Production and Methane Emissions. *J. Dairy Sci.* **2002**, *85*, 1509–1515. [CrossRef]
47. Palmquist, D.L.; Griinari, J.M. Milk Fatty Acid Composition in Response to Reciprocal Combinations of Sunflower and Fish Oils in the Diet. *Anim. Feed Sci. Technol.* **2006**, *131*, 358–369. [CrossRef]
48. Sun, X.; Guo, C.; Zhang, Y.; Wang, Q.; Yang, Z.; Wang, Z.; Wang, W.; Cao, Z.; Niu, M.; Li, S. Effect of Diets Enriched in N-6 or n-3 Fatty Acids on Dry Matter Intake, Energy Balance, Oxidative Stress, and Milk Fat Profile of Transition Cows. *J. Dairy Sci.* **2023**, *106*, 5416–5432. [CrossRef]
49. Kra, G.; Daddam, J.R.; Moallem, U.; Kamer, H.; Mualem, B.; Levin, Y.; Kočvarová, R.; Nemirovski, A.; Contreras, A.G.; Tam, J.; et al. Alpha-Linolenic Acid Modulates Systemic and Adipose Tissue-Specific Insulin Sensitivity, Inflammation, and the Endocannabinoid System in Dairy Cows. *Sci. Rep.* **2023**, *13*, 5280. [CrossRef]
50. Hassan, F.U.; Nadeem, A.; Li, Z.; Javed, M.; Liu, Q.; Azhar, J.; Rehman, M.S.U.; Cui, K.; Rehman, S.U. Role of Peroxisome Proliferator-Activated Receptors (PPARs) in Energy Homeostasis of Dairy Animals: Exploiting Their Modulation through Nutrigenomic Interventions. *Int. J. Mol. Sci.* **2021**, *22*, 12463. [CrossRef]
51. van Knegsel, A.T.M.; van den Brand, H.; Dijkstra, J.; Tamminga, S.; Kemp, B. Effect of Dietary Energy Source on Energy Balance, Production, Metabolic Disorders and Reproduction in Lactating Dairy Cattle. *Reprod. Nutr. Dev.* **2005**, *45*, 665–688. [CrossRef]
52. Ospina, P.A.; McArt, J.A.; Overton, T.R.; Stokol, T.; Nydam, D.V. Using Nonesterified Fatty Acids and β-Hydroxybutyrate Concentrations during the Transition Period for Herd-Level Monitoring of Increased Risk of Disease and Decreased Reproductive and Milking Performance. *Vet. Clin. N. Am. Food Anim. Pract.* **2013**, *29*, 387–412. [CrossRef] [PubMed]
53. Duffield, T. Subclinical Ketosis in Lactating Dairy Cattle. *Vet. Clin. N. Am. Food Anim. Pract.* **2000**, *16*, 231–253. [CrossRef]
54. Staples, C.R.; Burke, J.M.; Thatcher, W.W. Influence of Supplemental Fats on Reproductive Tissues and Performance Oflactating Cows. *J. Dairy Sci.* **1998**, *81*, 856–871. [CrossRef]
55. Chilliard, Y. Dietary Fat and Adipose Tissue Metabolism in Ruminants, Pigs, and Rodents: A Review. *J. Dairy Sci.* **1993**, *76*, 3897–3931. [CrossRef]
56. Mathews, A.T.; Rico, J.E.; Sprenkle, N.T.; Lock, A.L.; McFadden, J.W. Increasing Palmitic Acid Intake Enhances Milk Production and Prevents Glucose-Stimulated Fatty Acid Disappearance without Modifying Systemic Glucose Tolerance in Mid-Lactation Dairy Cows. *J. Dairy Sci.* **2016**, *99*, 8802–8816. [CrossRef]
57. Childs, S.; Lynch, C.O.; Hennessy, A.A.; Stanton, C.; Wathes, D.C.; Sreenan, J.M.; Diskin, M.G.; Kenny, D.A. Effect of Dietary Enrichment with Either N-3 or n-6 Fatty Acids on Systemic Metabolite and Hormone Concentration and Ovarian Function in Heifers. *Animal* **2008**, *2*, 883–893. [CrossRef]
58. Leroy, J.L.M.R.; Vanholder, T.; Van Knegsel, A.T.M.; Garcia-Ispierto, I.; Bols, P.E.J. Nutrient Prioritization in Dairy Cows Early Postpartum: Mismatch Between Metabolism and Fertility? *Reprod. Domest. Anim.* **2008**, *43*, 96–103. [CrossRef] [PubMed]
59. Freret, S.; Oseikria, M.; Le Bourhis, D.; Desmarchais, A.; Briant, E.; Desnoes, O.; Dupont, M.; Le Berre, L.; Ghazouani, O.; Bertevello, P.S.; et al. Effects of a N-3 Polyunsaturated Fatty Acid-Enriched Diet on Embryo Production in Dairy Cows. *Reproduction* **2019**, *158*, 71–83. [CrossRef]
60. Cerri, R.L.A.; Juchem, S.O.; Chebel, R.C.; Rutigliano, H.M.; Bruno, R.G.S.; Galvão, K.N.; Thatcher, W.W.; Santos, J.E.P. Effect of Fat Source Differing in Fatty Acid Profile on Metabolic Parameters, Fertilization, and Embryo Quality in High-Producing Dairy Cows. *J. Dairy Sci.* **2009**, *92*, 1520–1531. [CrossRef]
61. Amini, M.R.; Naserian, A.A.; Valizadeh, R.; Dirandeh, E.; Baghshahi, H.; Razavi, S.A. The Effects of Saturated and Polyunsaturated Fatty Acids on Reproductive Performance and Reproductive Hormonal Changes in Dairy Cows at the Transition Period. *World's Vet. J.* **2024**, *14*, 184–193. [CrossRef]
62. Zeng, X.; Li, S.; Liu, L.; Cai, S.; Ye, Q.; Xue, B.; Wang, X.; Zhang, S.; Chen, F.; Cai, C.; et al. Role of Functional Fatty Acids in Modulation of Reproductive Potential in Livestock. *J. Anim. Sci. Biotechnol.* **2023**, *14*, 24. [CrossRef] [PubMed]

63. Verma, A.K.; Mahla, A.S.; Chaudhari, R.K.; Singh, A.K.; Khatti, A.; Singh, S.K.; Dutta, N.; Singh, G.; Sarkar, M.; Kumar, H.; et al. Effect of Different Levels of N-3 Polyunsaturated Fatty Acids Rich Fish Oil Supplementation on the Ovarian and Endometrial Functions in the Goat (Capra Hircus). *Anim. Reprod. Sci.* **2018**, *195*, 153–161. [CrossRef] [PubMed]
64. Mattos, R.; Guzeloglu, A.; Badinga, L.; Staples, C.R.; Thatcher, W.W. Polyunsaturated Fatty Acids and Bovine Interferon-τ Modify Phorbol Ester-Induced Secretion of Prostaglandin F2α and Expression of Prostaglandin Endoperoxide Synthase-2 and Phospholipase-A2 in Bovine Endometrial Cells. *Biol. Reprod.* **2003**, *69*, 780–787. [CrossRef] [PubMed]