2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(2): 184-193.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj23
PII: S232245682400023-14

The Effects of Saturated and Polyunsaturated Fatty Acids on Reproductive Performance and Reproductive Hormonal Changes in Dairy Cows at the Transition Period

Mahmood Reza Amini[1], Abbas Ali Naserian[1]*, Reza Valizadeh[1], Essa Dirandeh[2], Hojjat Baghshahi[3], and Seyed Amin Razavi[4]

[1]*Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran*
[2]*Department of Animal Science, Sari Agricultural Sciences and Natural Resources University, Sari, Mazandaran, Iran*
[3] *Barij Essence Medicinal Plants Research Center, Kashan, Iran*
[4]*Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran*

*Corresponding author's Email: abasalin@yahoo.com

ORIGINAL ARTICLE
Received: April 02, 2024
Revised: April 24, 2024
Accepted: May 21, 2024
Published: June 25, 2024

ABSTRACT

Reproductive success is crucial in dairy farming as it heavily relies on the consumption of a complete mixed ration for the diet. The current study investigated the effects of adding saturated (SFA) and polyunsaturated fatty acids (PUFAs) to dairy cows' diets on reproductive performance and reproductive hormones during the transition period. A total of 30 Holstein dairy cows were randomly divided into three groups (10 animals in each group), based on parity and body condition score. The cows had an initial body weight of 567.5 ± 40.3 kg (mean ± SD), a body condition score of 3.5 ± 0.26 out of 5 (mean ± SD), and a parity of 1.7 ± 0.02 (mean ± SD). The control group received a balanced ration meeting all the nutrient requirements according to the National Research Council (NRC) guidelines. The SFA group received 1.4% of dry matter (DM) as palm oil (RumiFat®), while the omega group had 5% of DM as safflower (a source of n-6 fatty acids) added from 21 days before parturition to 21 days after, and 4% of DM as flaxseed (a source of n-3 fatty acids) added from 21 to 42 days after parturition. In the Omega group, estradiol concentration significantly increased on artificial insemination (AI) day (12.54 pg/mL). Additionally, serum prostaglandin F2-alpha concentration was significantly higher in the omega group (0.732 pg/mL on day 7 and 1.68 pg/mL on day 14) compared to other groups. The control group exhibited the highest progesterone concentrations at 14 and 21 days post-calving compared to the other groups, other groups. whereas the omega group highest concentration five days after AI. The omega group also showed a significantly higher mean number of follicles >10mm and larger ovulatory follicle diameter. Moreover, a higher percentage of pregnant cows at 120 days in milk, fewer open days, and lower service per conception were observed in the omega group compared to the other groups. In conclusion, supplementing dairy cows' diets with PUFAs during the transition period positively influenced ovarian function, hormone levels, and reproductive performance.

Keywords: Flaxseed, Follicle diameter, Omega, Ovarian function, Safflower

INTRODUCTION

Reproductive success is a crucial aspect of dairy farming that relies on a total mixed ration-based diet. Reproductive inefficiency can be caused by negative energy balance after parturition, leading farmers to add fat to the dairy cows' diet to optimize energy status (Castro et al., 2006). The supplementation of fat aims to increase energy intake while minimizing body cows' fat mobilization during the transition period, thereby reducing the incidence of early lactation disorders, such as fatty liver (Mirzaei et al., 2020). Modifying dietary fatty acid intake has the potential to improve reproductive measures in dairy cattle (Sammad et al., 2022). Of particular interest was the supplementation of long-chain fatty acids, especially polyunsaturated fatty acids (PUFAs, Nanas et al., 2023).

Supplementation of long-chain fatty acids, particularly polyunsaturated fatty acid (PUFA) has raised considerable interest due to the possible positive effect on reproductive performance (Castro et al., 2006). Vegetable oil obtained from oilseeds (soybean oil, safflower oil, sunflower oil, or flaxseed oil) or oilseeds themselves can be added to the ration as a source of fat that contains different fatty acids (Nanas et al., 2023).

Several reviews and a meta-analyze have examined the effects of PUFAs on ruminants (Leduc et al., 2017, Roque-Jiménez et al., 2021; Angeli et al., 2021; Veshkini et al., 2023). Ruminants cannot synthesize omega 3 (n-3) and omega 6 (n-6) fatty acids, making supplementation of these fatty acids essential in their diet (Mylostyvyi et al., 2021). The N-6 fatty acids, such as linoleic acid, can alter the fatty acid profile of phospholipids in cell membranes, increasing the proportion of arachidonic acids. This change had advantages for the synthesis of prostaglandins and eicosanoids (Dyall et al., 2022). Silvestre et al. (2011) demonstrated that feeding cows a Ca salt rich in n-6 fatty acids during late and early gestation increased fertility benefits during the breeding period (Silvestre et al., 2011). Another study found that

To cite this paper: Amini MR, Naserian AA, Valizadeh R, Dirandeh E, Baghshahi H, and Razavi SA (2024). The Effects of Saturated and Polyunsaturated Fatty Acids on Reproductive Performance and Reproductive Hormonal Changes in Dairy Cows at the Transition Period. *World Vet. J.,* 14(2): 184-193. DOI: https://dx.doi.org/10.54203/scil.2024.wvj23

supplementing dairy cows' diet with 10 g of docosahexaenoic acid (n-3) enhanced pregnancy per AI and pregnancy rate (Sinedino et al., 2017). According to Dirandeh et al. (2013), supplementing the diet with polyunsaturated fatty acids (n-3 and n-6) improved fertility in dairy cows. Moreover, Leduc et al. (2017) demonstrated the positive effect of certain fatty acids (PUFA) on the pituitary, ovaries, and uterus rather than improved energy status. It is indicated that certain fatty acids (n-3, n-6) can suppress PGF2α and are known to play a role in prostaglandins (PGF2α) synthesis. Therefore, supplementation of these fatty acids can result in a decrease in embryonic mortality due to a change in the profile of fatty acids in the diet and uterine synthesis of prostaglandins during early pregnancy.

The present study aimed to evaluate the effects of adding selective saturated fatty acids (SFA) and PUFA to dairy cows' diet on ovarian function, hormone concentration (serum prostaglandin F2-alpha [PGFM], progesterone, and estradiol concentrations) during the transition period.

MATERIALS AND METHODS

Ethical approval

All animals were treated in accordance with the regulations and guidelines set by the Iranian Council of Animal Care. The experiment received approval from the Iranian Ministry of Agriculture (experimental permission no. 1828).

Animal and experimental diets

The study was done at Mashhad University Agriculture Farm, Iran (from June to August 2020). A total of 30 Holstein dairy cows (10 animals in each group), consisting of 15 primiparous and 15 multiparous cows, were included in the study. The cows had an initial body weight of 567.5 ± 40.3 kg (mean ± SD), a body condition score of 3.5 ± 0.26 out of 5 (mean ± SD), and a parity of 1.7 ± 0.02 (mean ± SD). The cows were randomly assigned to one of three experimental treatments, each group consisted of 10 cows (5 primiparous and 5 multiparous). The experimental design was completely randomized, and the testing period spanned from 21 days before the expected calving date until 42 days after calving. The cows were housed in tie stalls (3x3x3 meters) and fed individually, with free access to fresh water throughout the testing period. The animals did not receive any vaccination during the experiment.

The cows were divided into three equal experimental groups based on parity, body condition score, and expected calving date. The control group received a balanced ration that met all the nutrient requirements according to the National Research Council (NRC) guidelines (Table 1, NRC, 2001). The other two treatment groups received different dietary supplements. The SFA group included the addition of 1.4% of dry matter (DM) palm oil (RumiFat®) to the diet, while omega group included the addition of 5% of DM safflower (a source of n-6 fatty acids) to the diet from 21 days before parturition to 21 days after parturition, and 4% of DM flaxseed (a source of n-3 fatty acids) from 21 days after parturition to 42 days after parturition. Milking was done twice a day (At 8 a.m. and 5 p.m.). The diets were fed as a total mixed ration (TMR) twice daily (0700 and 1700 hour) for *ad libitum* intake, with 10% of refusals on an as-fed basis.

Experimental procedure, sampling, and feeding

The cows were housed in a tie-stall barn that was randomly designed, with each group consisting of 10 cows, starting from 21 days before parturition. They were provided with a close-up diet from the first day after parturition until 21 days after parturition, followed by a fresh cow's diet until 21 days after parturition, and final diet from 21 days after parturition to 42 days after parturition (Table 1). The total mixed rations were sampled weekly, pooled monthly, and analyzed in the laboratory of Mashhad University Agriculture for dry matter content using a drying oven method (Mylostyvyi et al., 2021). The samples (500 g) were also analyzed for crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) according to the official methods of analysis by the Association of Official Analytical Chemists (AOAC, Mylostyvyi et al., 2021).

Blood sampling and analysis

Blood samples (10 ml) were collected from all cows 7, 14, and 21 days after parturition, on the day of AI, and 5 days after AI. The samples were collected via coccygeal venipuncture and placed in Vacutainer tubes (BD Vacutainer Systems, Plymouth, UK) without anticoagulants. After allowing the blood to coagulate for two hours, it was centrifuged for 15 minutes at 3500 rpm. The serum was then transferred to microtubes and frozen at -20°C for further laboratory analysis. The concentrations of progesterone (ng/mL), estradiol (pg/mL), and PGFM (pg/mL), in the serum were determined using ELISA kits (progesterone and estradiol: Diaplus, North York, Ontario, Canada, Intra- and intra-assay coefficients of variation were < 5% (PGFM: Cayman Chemical, Ann Arbor, MI, USA). The sensitivity of the PGFM assay was 0.02 ng/mL.

Table 1. Ingredient and chemical composition of experimental diets of Holstein dairy cows during the transition period (21 days before calving to 42 days after calving)

Item	Close up period[1]			Fresh cow period[2]			Early lactation period[3]		
	Control	SFA	Omega (Safflower seed)	Control	SFA	Omega (Safflower seed)	Control	SFA	Omega (Flaxseed)
Ingredient (Percentage of dry matter)									
Alfalfa hay	21.22	21.13	21.14	27.24	27.38	27.38	13.47	13.46	13.46
Corn silage	29.12	29	29.01	17.85	17.95	17.95	26.93	26.92	26.92
wheat straw	10.04	10	10.01	-	-	-	-	-	-
Barley, rolled	19.72	18.3	14.68	5.57	4.15	0.26	5.96	5.96	5.96
Corn	-	-	-	24.26	23.85	23.85	25.63	24.31	21.77
Soybean meal	4.25	4.23	4.23	9.54	9.59	9.59	13.12	13.42	11.15
Corn gluten meal	-	-	-	2.38	2.39	2.39	2.98	2.98	3
Canola meal	4.3	4.72	2.75	2.76	3.55	1.54	2.98	2.98	3
Meat meal	1.79	1.79	1.79	-	-	-	-	-	-
Bran	8.74	8.63	10.62	2.73	2.74	2.74	6.23	5.96	8.19
Sugar beet pulp	-	-	-	5.4	4.67	6.68	-	-	-
Calcium salt of palm fatty acid	-	1.38	-	-	1.45	-	-	1.31	-
Safflower seed	-	-	4.96	-	-	5.34	-	-	-
Flaxseed	-	-	-	-	-	-	-	-	3.85
Calcium bicarbonate	0.29	0.28	0.28	0.43	0.43	0.43	0.42	0.42	0.42
Dicalcium phosphate	-	-	-	-	-	-	0.12	0.12	0.12
Sodium bicarbonate	-	-	-	0.55	0.55	0.55	0.62	0.62	0.62
Magnesium oxide	-	-	-	0.18	0.18	0.18	0.23	0.23	0.23
Vitamins/minerals[4]	0.53	0.53	0.53	0.92	0.92	0.92	0.88	0.88	0.88
Vitamin E and Selenium Supplement[5]	0.15	0.15	0.15	0.15	0.15	0.15	0.23	0.23	0.23
Salt	-	-	-	0.18	0.18	0.18	0.19	0.19	0.19
Nutrient composition (Dry matter basis)									
NEL,[6] Mcal/kg	1.58	1.62	1.6	1.55	1.59	1.58	1.61	1.65	1.63
CP, % of DM	13.8	13.8	13.8	16.2	16.2	16.2	15.7	15.8	15.9
NFC, % of DM	36.5	35.6	33.4	46.1	45.1	44.1	45.2	44	41.8
Fatty acids, % of DM	2.8	4.1	4.1	2.9	4.1	4.1	2.7	4.1	4
NDF, % of DM	42.4	42	44	30.1	29.9	31.1	31.5	31.2	33.3
ADF, % of DM	26.4	26.3	27.8	18.3	18.3	18.6	20.2	20.2	22
Ca, % of DM	0.7	0.7	0.7	0.6	0.6	0.6	0.7	0.7	0.7
P, % of DM	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4

[1] Control: no fat supplement; SFA: Saturated fatty acid supplement; Omega: Polyunsaturated fatty acids (PUFAs) supplement (from 21 d before predicted calving until calving).
[2] Control: no fat supplement; SFA: Saturated fatty acid supplement; Omega: PUFAs fatty acid supplement (from calving until 21 d postpartum).
[3] Control: no fat supplement; SFA: Saturated fatty acid supplement; Omega: PUFAs fatty acid supplement (from 21 to 42 d postpartum).
[4] Contained 68,679 mg/kg Ca; 30,000 mg/kg P; 40,000 mg/kg Na; 20,700 mg/kg Mg; 1,840 mg/kg of Cu; 5,000 mg/kg of Mn; 100 mg/kg of Co; 10,395 mg/kg of Zn; 100 mg/kg of Se; 2 mg/kg of organic Se; 120 mg/kg of I; 1,000 kIU/kg of vitamin A; 500 kIU/kg of vitamin D; 1 kIU/kg of vitamin E; and 1,000 mg/kg of antioxidants.
[5] Contained 300 mg/kg of Se; 11,000 mg/kg of vitamin E; and 400 mg/kg of antioxidants.
[6] Calculated based on the DMI of 11.5 kg per day and using NRC software (version 2001).

To cite this paper: Amini MR, Naserian AA, Valizadeh R, Dirandeh E, Baghshahi H, and Razavi SA (2024). The Effects of Saturated and Polyunsaturated Fatty Acids on Reproductive Performance and Reproductive Hormonal Changes in Dairy Cows at the Transition Period. *World Vet. J.*, 14(2): 184-193. DOI: https://dx.doi.org/10.54203/scil.2024.wvj23

Body weight and body condition score

The body weight of all cows in the study was measured, and their body condition score (BCS) was evaluated at the beginning of the study. The BCS was assigned to each cow on a scale of 1 to 5, with the increase of 0.25, according to the method described by Edmonson et al. (1989).

Reproductive management

The cows in the study were synchronized using two injections of $PGF_{2\alpha}$ (150 mg cloprostenol sodium, syva, Spain) administered 14 days apart, starting at 30 ± 3 and 44 ± 3 days in milk (DIM) postpartum. The day after the second $PGF_{2\alpha}$ injection, the cows' uterus and ovaries were scanned using an ultrasound linear rectal transducer (Ecogra, 7.5 MHz transrectal linear transducer, Aloca Co., Ltd., Tokyo, Japan) for three days (45 ± 3 DIM, 46 ± 3 DIM, and 47 ± 3 DIM) to evaluate ovarian function (number of follicles and diameters). The follicles were categorized into three diameter classes (Ultrasound device measurement was used to categorize the follicles) including small (3.0 to 4.9 mm), medium (5.0 to 9.9 mm), and large (≥ 10 mm). The diameter of each follicle was measured as the average length and width of the antrum (Dyall et al., 2022). Cows that exhibited estrus after the second $PGF_{2\alpha}$ injection were artificially inseminated, while cows that did not show estrus were inseminated during their subsequent estrus. Artificial insemination was performed by a technician using commercially available frozen-thawed semen (USA). Pregnancy information, open days, and insemination for pregnancy were recorded using livestock information software (Modiran software version 2.3, Iran).

Statistical analysis

Data were analyzed using the MIXED procedure of SAS software (version 9.4, 2018, SAS Institute Inc., Cary, NC, USA). The statistical model included treatment, parity, and their interaction as fixed effects. The individual cow was considered as the experimental unit. The repeated measures of blood parameters were analyzed using the MIXED procedure with repeated measures. The random residual error was also included in the model.

$Y_{ijkl} = \mu + D_i + T_j + C_k + DT_{ij} + \varepsilon_{ijkl}$

Where Y_{ijkl} = the dependent variable, μ = the overall mean, D_i =the effect of diet, T_j = the effect of time of sampling, C_k = the effect of cow, DT_{ij} = the interactions between diet (D) and time (T), and ε_{ijkl} = the random residual error.

The best fitting covariance structure for the data was chosen based on the Schwartz Bayesian Information Criterion (BIC). The compound symmetry (CS) structure was chosen as it had the lowest BIC among the considered structures, which also included first-order autoregressive (AR1), heterogeneous first-order autoregressive (ARH1), and unstructured (UN) structures.

The significance level for the analysis was set at $p < 0.05$, with $p < 0.1$ considered as a trend. The Chi-square test with PROC FREQ was used to examine the number of pregnant cows after the first AI, as well as the overall number of pregnant cows. This allowed for comparisons of all possible dietary treatment pairs. The number of AI and open days were examined using the Kruskal-Wallis test with PROC NPAR1WAY, and the Mann-Whitney W test was used to compare the medians of the three groups two by two (Wilcoxon). The Dunn-Sidák multiple comparison method was used to adjust p-values derived from all tested comparisons.

RESULTS

Serum estradiol concentration

The results of estradiol concentration were indicated in Figure 1. The Omega (when supplemented with flaxseed) group had the highest estradiol concentration (12.54 pg/mL), while the SFA group had the lowest (8.9 pg/mL) estradiol concentration on AI day ($p < 0.05$).

Serum prostaglandin concentration

The effects of SFA and PUFAs on PGFM concentration at 7 and 14 days after parturition were indicated in Figure 2. Experimental groups showed a significant effect on PGFM concentration on day 7 and day 14 ($p < 0.05$). The Omega (when supplemented with safflower) group had the highest PGFM concentration on both days (0.732 pg/mL on day 7 and 1.68 pg/mL on day 14), while the control group had the lowest concentration on both days (0.32 pg/mL on day 7 and 0.94 pg/mL on day 14).

Serum progesterone concentration

The effects of SFA and PUFAs on serum progesterone concentration at 14 and 21 days after parturition, AI day, and 5 days after AI were indicated in Figure 3. The control group had the highest progesterone concentration (1.15 pg/mL) at 14 days after parturition compared to the SFA and Omega (safflower) groups ($p < 0.05$). The Omega

To cite this paper: Amini MR, Naserian AA, Valizadeh R, Dirandeh E, Baghshahi H, and Razavi SA (2024). The Effects of Saturated and Polyunsaturated Fatty Acids on Reproductive Performance and Reproductive Hormonal Changes in Dairy Cows at the Transition Period. *World Vet. J.,* 14(2): 184-193. DOI: https://dx.doi.org/10.54203/scil.2024.wvj23

(safflower) group had the lowest concentration (0.5 pg/mL) at 14 days after parturition. At 21 days after parturition, the control group had the highest progesterone concentration (1.65 pg/mL), while the SFA group had the lowest (0.76 pg/mL, $p < 0.05$). Five days after AI, the Omega (flaxseed) group had the highest progesterone concentration (2.57 pg/mL), while the control group had the lowest concentration (0.85 pg/mL, $p < 0.05$).

Reproductive performance, number and diameter of the ovulatory follicle

Effect of SFA and PUFAs on the mean number of follicles, diameter of the ovulatory follicle, and reproductive performance during the transition period were presented in Tables 2 and 3. The Omega group had a higher mean number of follicles >10 mm and a larger diameter of the ovulatory follicle ($p < 0.05$). The percentage of pregnant cows until 120 DIM was higher in the Omega group ($p < 0.05$), and they also had fewer open days ($p = 0.004$). Additionally, the Omega group had a lower service per conception compared to the other groups ($p = 0.002$).

Table 2. Means number of follicles, the diameter of the ovulatory follicle of adults Holstein dairy cows supplemented with saturated fatty acids and polyunsaturated fatty acids during the transition period (21 days before calving to 42 days after calving)

Variable \ Treatments [1]	Control	SFA	Omega	SD	P-value
Follicle no.					
<5 mm	7.00	6.91	7.1	0.43	0.90
5-10 mm	3.30	3.49	3.47	0.28	0.81
>10 mm	0.92[b]	0.60[c]	0.96[a]	0.06	<0.01
Diameter of the ovulatory follicle (mm)	13.6[b]	12.2[c]	15.2[a]	0.44	<0.05

[1]Control: No fat supplement; SFA: Saturated fatty acid supplement; Omega: Poly unsaturated fatty acids (PUFAs) fatty acid supplement. [a,b,c] Means within a row with different lowercase superscripts differ ($p < 0.05$). SD: Standard deviation.

Table 3. Reproductive performances of adults Holstein dairy cows supplemented with saturated fatty acids and polyunsaturated fatty acids during the transition period (21 days before calving to 42 days after calving)

Variable \ Treatments [1]	Control	SFA	Omega	SD	P-value
Pregnant cows until 120 DIM (%)	42.85 [c]	66.60 [b]	85.71 [a]	0.13	0.001
Open days (day)	108.28[a]	111.5[a]	86.14[b]	3.2	0.004
Service per conception	2.28[a]	1.89[b]	1.50[c]	0.08	0.002

[1]Control: No fat supplement; SFA: Saturated fatty acid supplement; Omega: Poly unsaturated fatty acids (PUFAs) supplement. DIM: Days in milk. [a,b,c] Mean within a row with different lowercase superscripts differ $p < 0.05$. SD: Standard deviation.

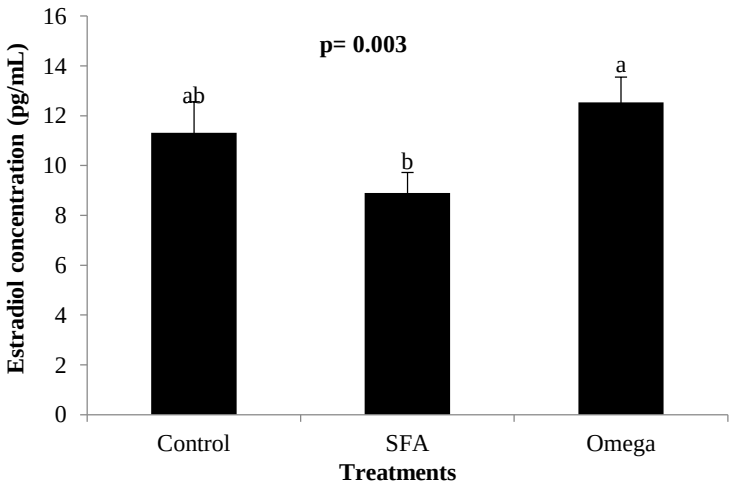

Figure 1. Mean (± SD) serum estradiol concentration (pg/mL) on artificial insemination day in Holstein dairy cow in the experimental groups from 21 days before to 42 days after calving. The groups including control, saturated fatty acids group (SFA), and polyunsaturated fatty acids group (Omega). [a, b] Mean different lowercase letters shows significant differences between the groups $p < 0.05$.

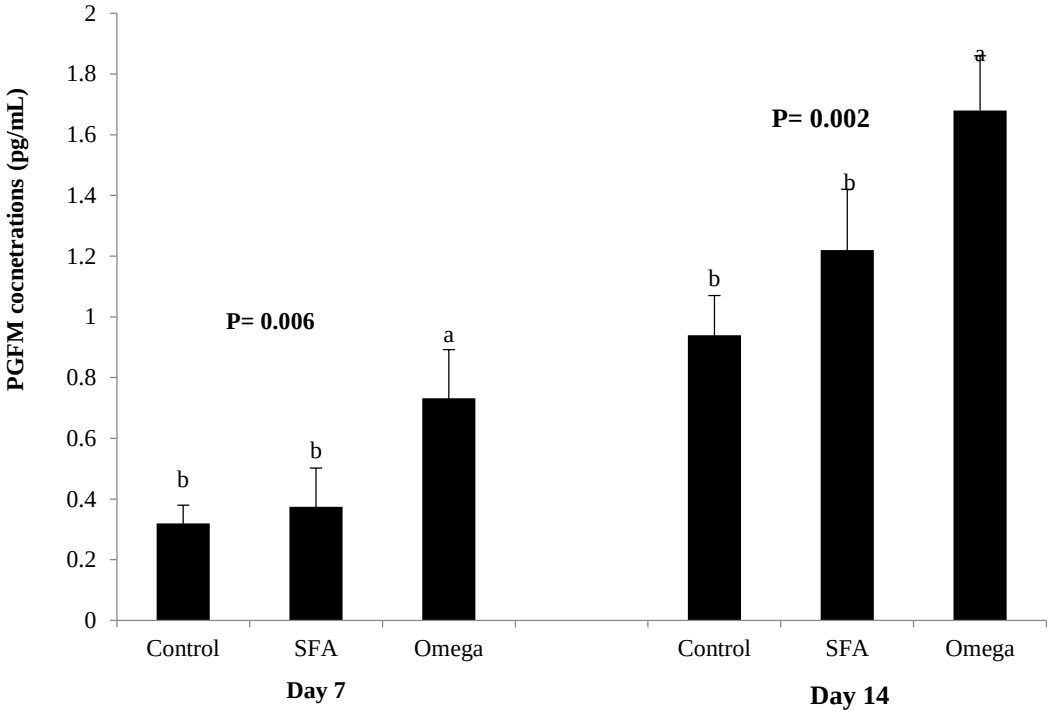

Figure 2. Mean (± SD) serum PGFM concentration (pg/mL) concentration on days 7 and 14 after parturition Holstein dairy cow in the experimental groups from 21 days before to 42 days after calving. The groups including control, saturated fatty acids group (SFA), and polyunsaturated fatty acids group (Omega). [a, b] Mean different lowercase letters shows significant differences between the groups $p < 0.05$.

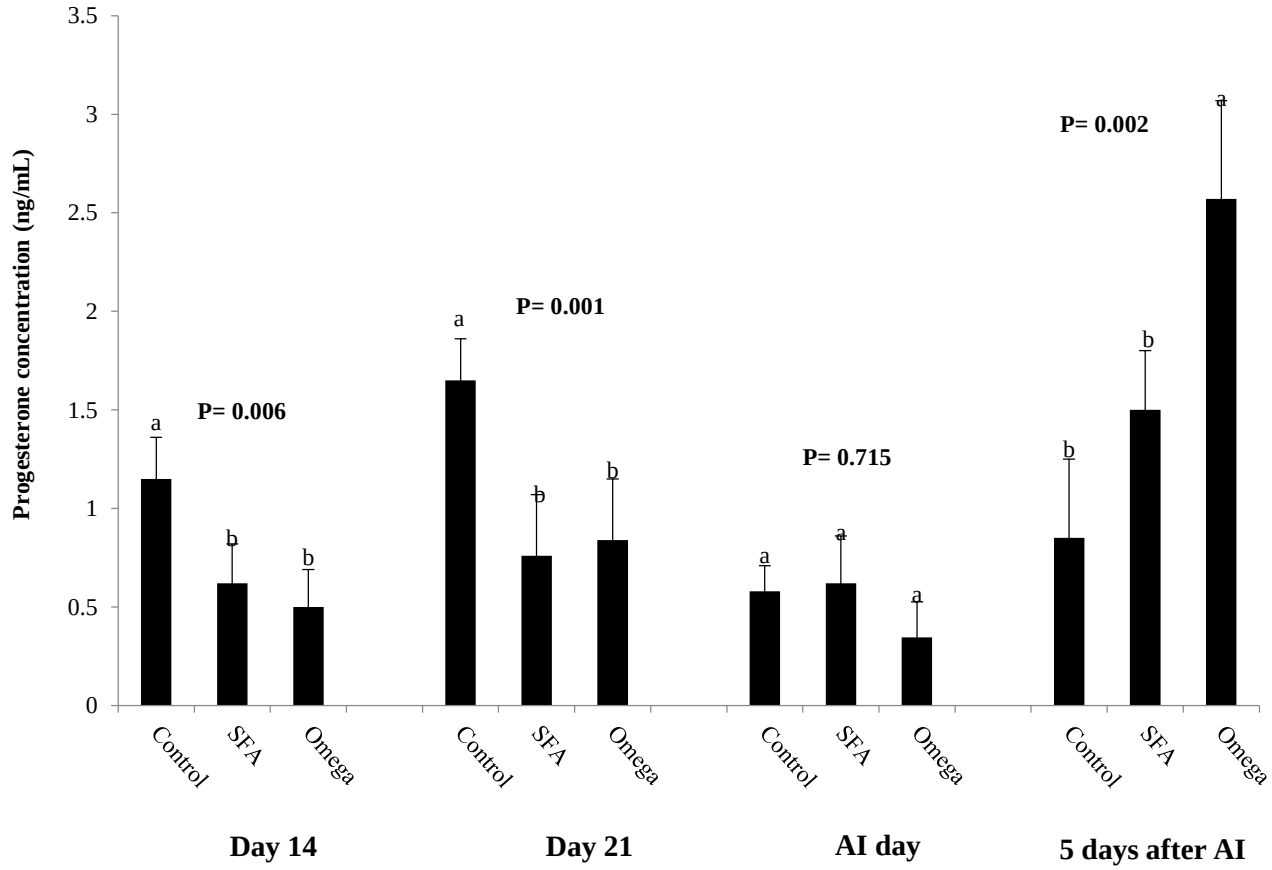

Figure 3. Mean (± SD) serum Progesterone concentration (ng/mL) on days 14 and 21 after parturition, artificial insemination day, and 5 days after artificial insemination Holstein dairy cow in the experimental group. The groups including control, saturated fatty acids group (SFA), and polyunsaturated fatty acids group (Omega). [a, b] Mean different lowercase letters shows significant differences between the groups $p < 0.05$.

DISCUSSION

An increase in estradiol concentration is associated with the initiation of estrus and can have positive effects on the process of AI (Tippenhauer et al., 2023). In this study, the group supplemented with safflower (source of omega 6) had the highest estradiol concentration on AI day, it is indicating that supplementation of PUFA to Holstein dairy cows could enhance the AI process and result in a higher conception rate.

Prostaglandins, particularly $PGF_{2\alpha}$, play a crucial role in luteolysis in ruminants (Greco et al., 2018). The precursor of PGFM is arachidonic acid, formed from the elongation and desaturation of linoleic acid (Tallima et al., 2018). PUFA, such as those found in safflower, can affect the synthesis of $PGF_{2\alpha}$ and progesterone (Gulliver et al., 2012). The safflower group in this study had higher concentrations of PGFM at 7 and 14 days after parturition compared to other groups. The current study results align with Caldari-Torres et al.'s (2006) study, which demonstrated that an increased ratio of n-6 to n-3 fatty acids in the culture media led to elevated synthesis of $PGF_{2\alpha}$ (Caldari-Torres et al., 2006). This indicated that modifying the fatty acid composition of the diet may result in the release of PG from the endometrium and potentially mitigate luteolytic signals. However, contrary to current study findings, Dirandeh et al. (2013) and Petit et al. (2004) found that increased n-3 fatty acids suppressed the release of $PGF_{2\alpha}$ following an oxytocin challenge (Petit et al., 2004; Dirandeh et al., 2013). This increase in PGFM may influence the lifespan of the corpus luteum and result in an earlier return to estrus. Silvestre et al. (2011) observed an increase in the pregnancy per AI ratio and a decrease in pregnancy loss among cows fed Ca salts enriched with PUFA during the breeding period (Silvestre et al., 2011). In the current study, the Omega group had a higher number of pregnant cows, fewer open days, and a lower service per conception compared to other groups. These results indicate that PUFA supplementation may improve reproductive outcomes in dairy cows.

Furthermore, Demetrio et al. (2007) and Lopes et al. (2009) found that dairy cows supplied with n-3 PUFA had higher plasma progesterone levels (Demetrio et al., 2007; Lopes et al., 2009). Moreover, cows treated with PUFA exhibited a higher number of pregnant cows on the first timed artificial insemination (TAI), improved conception rates, and enhanced overall reproductive performance. Stevenson et al. (2006) and Chebel et al. (2010) discovered that $PGF_{2\alpha}$ secretion may have been reduced during pregnancy detection in cows fed flaxseed as an n-3 supplement. In accordance with the findings of Castro et al. (2006), the group that received PUFA-rich vegetable oil exhibited higher plasma progesterone levels and a greater number of pregnant cows on the first AI. Additionally, these animals had a lower incidence of AI. The omega group (PUFA) had the highest progesterone levels 5 days after AI, and a higher number of cows in this group became pregnant. This aligns with previous studies that had shown higher plasma progesterone levels and improved reproductive outcomes in cows treated with PUFA (Stevenson et al., 2006; Chebel et al., 2010). It had been suggested that the production of $PGF_{2\alpha}$ may have been reduced during pregnancy recognition in cows that were fed flaxseed (a source of omega 3). Progesterone plays a crucial role in preparing the uterus for embryo implantation and supporting pregnancy by nourishing the conceptus (Castro et al., 2006).

The supplementation of PUFA in the diets of dairy cows had the potential to improve fertility. Several studies have indicated that PUFA may enhance fertility by influencing follicular growth and ovulation (Robinson et al., 2002; Libera et al., 2020). It is demonstrated that cows fed with PUFA exhibited larger follicle diameters and an increased number of follicles (Robinson et al., 2002; Kabirian Moghadam et al., 2020; 2023). In the current study, the authors observed a higher number of follicles and a larger diameter of the ovulatory follicle in the group that received PUFA.

In this study, safflower (a source of n-6) was incorporated into the cows' diets from 21 days before parturition to 21 days after parturition. This was done because safflower is known to increase $PGF_{2\alpha}$ concentration and potentially attenuate luteolytic signals, thereby influencing the lifespan of the corpus luteum and resulting in an earlier return to estrus. Additionally, the inclusion of flaxseed (a source of n-3) from 21 days after parturition to 42 days after parturition was associated with higher plasma progesterone levels. This increase in progesterone may lead to improved reproductive performance, including an increased number of pregnant cows and a higher rate of successful conception.

CONCLUSION

In this study found that supplementing a source of n-6 fatty acids before and after parturition and a source of n-3 fatty acids after parturition can have positive effects on reproductive performance in dairy cows. Safflower (source of n-6) was found to increase prostaglandin concentration and potentially mitigate luteolytic signals, while flaxseed (source of n-3) resulted in higher plasma progesterone levels and improved reproductive outcomes such as the number of pregnant cows and service per conception. The selective supplementation of n-6 and n-3 fatty acids based on the follicular cycle of dairy cows could be beneficial for their reproductive performance. As a suggestion, further studies need to evaluate the effects of PUFA in different pregnancy and lactation periods in dairy cows.

DECLARATIONS

Funding

No external, commercial or academic funding source was received for present study.

Acknowledgments

The authors wish to acknowledge technical support from the Ferdowsi University of Mashhad and the Centre of Excellence for Animal Science.

Availability of data and materials

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

Mahmood Reza Amini, Abasali Naserian and Reza valizadeh planned the experiment. Seyed Amin Razavi and Essa Dirandeh interpreted and analyzed the data the write the manuscripts and Hojjat Baghshahi revised the paper. All authors read and approved the final edition of the manuscript.

Ethical considerations

The authors confirm that all authors have reviewed and submitted the manuscript to this journal for the first time.

Competing interests

The authors declare no conflict of interest.

REFERENCES

Angeli E, Barcarolo D, Ribas L, Marelli BE, Roskopf PM, Salado EE, Ortega HH, and Hein GJ (2021). Relevant aspects of dietary n-3 polyunsaturated fatty acids in the adaptation of dairy cattle to the transition period. Livestock Science, 244: 104346. DOI: https://www.doi.org/10.1016/j.livsci.2020.104346

Castro T, Caldari-Torres C, Rodriguez-Sallaberry C, Greene E, and Badinga L (2006). Differential effects of n-3 and n-6 fatty acids on prostaglandin F2α production by bovine endometrial cells. Journal of Dairy Science, 89(3): 971-977. DOI: https://www.doi.org/10.3168/jds.S0022-0302(06)72162-2

Chebel RC, Al-Hassan MJ, Fricke PM, Santos JEP, Lima JR, Martel CA, Stevenson JS, Garcia R, and Ax RL (2010). Supplementation of progesterone via controlled internal drug release inserts during ovulation synchronization protocols in lactating dairy cows. Journal of Dairy Science, 93(3): 922-931. DOI: https://www.doi.org/10.3168/jds.2009-2301

Demetrio D, Santos R, Demetrio C, and Vasconcelos JLM (2007). Factors affecting conception rates following artificial insemination or embryo transfer in lactating Holstein cows. Journal of Dairy Science, 90(11): 5073-5082. DOI: https://www.doi.org/10.3168/jds.2007-0223

Dirandeh E, Towhidi A, Pirsaraei ZA, Adib Hashemi F, Ganjkhanlou M, Zeinoaldini S, Rezaei Roodbari A, Saberifar T, and Petit HV (2013). Plasma concentrations of PGFM and uterine and ovarian responses in early lactation dairy cows fed omega-3 and omega-6 fatty acids. Theriogenology, 80(2): 131-137. DOI https://www.doi.org/10.1016/j.theriogenology.2013.03.012

Dyall SC, Balas L, Bazan NG, Brenna JT, Chiang N, da Costa Souza F, Dalli J, Durand T, Galano JM, Lein PJ et al. (2022). Polyunsaturated fatty acids and fatty acid-derived lipid mediators: Recent advances in the understanding of their biosynthesis, structures, and functions. Progress in Lipid Research, 86: 101165. DOI: https://www.doi.org/10.1016/j.plipres.2022.101165

Edmonson A, Lean I, Weaver L, Farver T, and Webster G (1989). A body condition scoring chart for Holstein dairy cows. Journal of Dairy Science, 72: 68-78. DOI: https://www.doi.org/10.3168/jds.S0022-0302(89)79081-0

Gulliver C, Friend M, King B, and Clayton EH (2012). The role of omega-3 polyunsaturated fatty acids in reproduction of sheep and cattle. Animal Reproduction Science, 131(1-2): 9-22. DOI: https://www.doi.org/10.1016/j.anireprosci.2012.02.002

Greco L, Neto JN, Pedrico A, Lima FS, Bisinotto RS, Martinez N, Ribeiro ES, Thatcher WW, Staples CR, and Santos JEP (2018). Effects of altering the ratio of dietary n-6 to n-3 fatty acids on spontaneous luteolysis in lactating dairy cows. Journal of Dairy Science, 101(11): 10536-10556. DOI: https://www.doi.org/10.3168/jds.2018-15065

Kabirian Moghadam S, Hajimohammadi A, Nazifi S, Razavi SA, and Rowshan-Ghasrodashti A (2020). Effects of probiotic and chromium-methionine on thyroid hormones, total protein, zinc, and weight gain in dairy Holstein calves

during the weaning period. Iranian Journal of Veterinary Medicine, 14(2): 177-188. DOI: https://www.doi.org/10.22059/ijvm.2019.283302.1004997

Kabirian Moghadam S, Razavi SA, Hajimohammadi A, Nazifi S, and Rowshan-Ghasrodashti A (2023). Evaluating oxidative stress and immune response by adding probiotic and chromium methionine during weaning period in dairy calves. Comparative Clinical Pathology, 32: 117-124. DOI: https://www.doi.org/10.1007/s00580-022-03421-6

Leduc M, Létourneau-Montminy MP, Gervais R, and Chouinard PY (2017). Effect of dietary flax seed and oil on milk yield, gross composition, and fatty acid profile in dairy cows: A meta-analysis and meta-regression. Journal of Dairy Science, 100(11): 8906-8927. DOI: https://www.doi.org/10.3168/jds.2017-12637

Lopes CN, Scarpa AB, Cappellozza BI, Cooke RF, and Vasconcelos JLM (2009). Effects of rumen-protected polyunsaturated fatty acid supplementation on reproductive performance of Bos indicus beef cows. Journal of Animal Science, 87(12): 3935-3943. DOI: https://www.doi.org/10.2527/jas.2009-2201

Libera K, Włodarek J, Warzych E, Cieślak A, Szumacher-Strabel M, Jankowiak T, and Lechniak D (2020). Reproductive performance of dairy cows fed a diet supplemented with n-3 polyunsaturated fatty acids-A review. Annals of Animal Science, 20(4): 1169-1183. DOI: https://www.doi.org/10.2478/aoas-2020-0040

Martinez D, Isabel B, Cabezas A, and Jimeno V (2019). Vegetable oils rich in polyunsaturated fatty acids supplementation of dairy cows' diets: Effects on productive and reproductive performance. Animals, 9(5): 205. DOI: https://www.doi.org/10.3390/ani9050205

Mirzaei A, Hajimohammadi A, Badiei K, Pourjafar M, Naserian AA, and Razavi SA (2020). Effect of dietary supplementation of bentonite and yeast cell wall on serum endotoxin, inflammatory parameters, serum, and milk aflatoxin in high-producing dairy cows during the transition period. Comparative Clinical Pathology, 29: 433-440. DOI: https://www.doi.org/10.1007/s00580-019-03074-y

Mylostyvyi R, Sejian V, Izhboldina O, Kalinichenko O, Karlova L, Lesnovskay O, Begma N, Marenkov O, Lykhach V, Midyk S et al. (2021). Changes in the spectrum of free fatty acids in blood serum of dairy cows during a prolonged summer heat wave. Animals, 11(12): 3391. DOI: https://www.doi.org/10.3390/ani11123391

Nanas I, Dokou S, Athanasiou LV, Dovolou E, Chouzouris TM, Vasilopoulos S, Grigoriadou K, Giannenas I, and Amiridis GS (2023). Feeding flaxseed and lupins during the transition period in dairy cows: Effects on production performance, fertility and biochemical blood indices. Animals, 13(12): 1972. DOI: https://www.doi.org/10.3390/ani13121972

National research council (NRC) (2001). Nutrient requirements of dairy cattle, 7th Edition. National Academ Press., Washington, USA. P:217-289

Petit H, Germiquet C, and Lebel D (2004). Effect of feeding whole, unprocessed sunflower seeds and flaxseed on milk production, milk composition, and prostaglandin secretion in dairy cows. Journal of Dairy Science, 87: 3889-3898. DOI: https://www.doi.org/10.3168/jds.S0022-0302(04)73528-6

Roque-Jiménez JA, Rosa-Velázquez M, Pinos-Rodríguez JM, Vicente-Martínez JG, Mendoza-Cervantes G, Flores-Primo A, Lee-Rangel HA, and Relling AE (2021). Role of long chain fatty acids in developmental programming in ruminants. Animals, 11(3): 762. DOI: https://www.doi.org/10.3390/ani11030762

Silvestre F, Carvalho T, Francisco N, Santos JEP, Staples CR, Jenkins TC, and Thatcher WW (2011). Effects of differential supplementation of fatty acids during the peripartum and breeding periods of Holstein cows: I. Uterine and metabolic responses, reproduction, and lactation. Journal of Dairy Science, 94(1): 189-204. DOI: https://www.doi.org/10.3168/jds.2010-3370

Sinedino LD, Honda PM, Souza LR, Lock AL, Boland MP, Staples CR, Thatcher WW, and Santos JEP (2017). Effects of supplementation with docosahexaenoic acid on reproduction of dairy cows. Reproduction, 153: 707-723. DOI: https://www.doi.org/10.1530/REP-16-0642

Sammad A, Khan MZ, Abbas Z, Hu L, Ullah Q, Wang Y, Zhu H, and Wang Y (2022). Major nutritional metabolic alterations influencing the reproductive system of postpartum dairy cows. Metabolites, 12(1): 60. DOI: https://www.doi.org/10.3390/metabo12010060

Stevenson JS, Pursley JR, Garverick HA, Fricke FM, Kesler DJ, and Ottobre JS (2006). Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. Journal of Dairy Science, 89(7): 2567-2578. DOI: https://www.doi.org/10.3168/jds.S0022-0302(06)72333-5

Tippenhauer C, Plenio JL, Heuwieser W, and Borchardt S (2023). Association of activity and subsequent fertility of dairy cows after spontaneous estrus or timed artificial insemination. Journal of Dairy Science, 106(6): 4291-4305. DOI: https://www.doi.org/10.3168/jds.2022-22057

Tallima H and El Ridi R (2018). Arachidonic acid: Physiological roles and potential health benefits-A review. Journal of Advanced Research, 11: 33-41. DOI: https://www.doi.org/10.1016/j.jare.2017.11.004

Robinson RS, Pushpakumara PG, Cheng Z, Peters AR, Abayasekara DR, and Wathes DC (2002). Effects of dietary polyunsaturated fatty acids on ovarian and uterine function in lactating dairy cows. Reproduction, 124(1): 119-131. DOI: https://www.doi.org/10.1530/rep.0.1240119

Veshkini A, Ceciliani F, Bonnet M, and Hammon HM (2023). Review: Effect of essential fatty acids and conjugated linoleic acid on the adaptive physiology of dairy cows during the transition period. Animal, 17(2): 100757. DOI: https://www.doi.org/10.1016/j.animal.2023.100757