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Effect of omega-3 polyunsaturated fatty acid supplementation on oxidative stress parameters and sex hormone levels of modern genotype sows

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Abstract

Background: Sows are exposed to severe stress and hormonal challenges during their whole productive life. As polyunsaturated fatty acids play an important role in immune and reproductive functions, with a better understanding of their role in breeding sows' nutrition, improved performance and more sustainable pig production can be achieved.

Objectives: In this study, we investigated the effects of omega-6 and omega-3 fatty acid supplementation on the antioxidant status and hormone levels of sows.

Methods: A total of 48 Danish Large white × Danish Landrace sows were supplemented either with sunflower oil (SO) as a control group or with fish oil (FO) as experimental group at the same dose of 10 g/kg feed. Blood samples were collected on day 14 of lactation, 5 days after weaning (insemination), and 30 days after insemination. To estimate antioxidant and reproductive effects, the amounts of reduced glutathione (GSH), thiobarbituric acid reactive substance, the activity of glutathione peroxidase (GPx), serum 17β oestradiol (E2), progesterone (P4), and 6-keto prostaglandin F1α (6-keto PGF1α) levels were investigated.

Results: FO-based supplementation increased GPx activity on day 14 of lactation. Five days after weaning, the concentration of GSH in FO-fed sows was significantly higher than that in SO-fed sows. The E2 content of blood was significantly lower in the experimental group than in the control group for two of the three examined periods (day 14 of lactation and 30 days after insemination), whereas P4 levels were significantly higher in the experimental group 5 days after weaning. We found that 6-keto PGF1α levels were systematically lower in the experimental group throughout the trial.

Conclusions: This study provides evidence of the major impact of omega-6 and -3 fatty acids on the tested hormone levels, which serve as precursors for the production of E2 and P4 but have an opposite effect on PGF2α production.

KEYWORDS
fish oil, oestrogen, omega-3 fatty acids, oxidative stress, progesterone, prostaglandin

1 | INTRODUCTION

The animals in swine farms, particularly breeding sows, are continuously exposed to severe immunosuppression. Weaning, insemination, nutrition, and location changes are stress factors that impair animal performance through increased susceptibility to diseases and reproductive failure. Immunosuppression must be modulated because it directly correlates with the economic benefits of livestock production (Lee & Kang, 2019). Polyunsaturated fatty acids (PUFAs) are energy sources and membrane components that regulate the expression of genes involved in several biological processes (Simopoulos, 2002). Among PUFAs, linoleic acid (LA; C18:2, omega-6) and α-linolenic acid (ALA; C18:3, omega-3) are essential for swine because they only are acquired from the diet. The chemistry, physiology, and metabolism of essential fatty acids are well documented in the scientific literature (Sprecher, 2000). It is known that after absorption, omega-6 and omega-3 fatty acids are transported either in the phospholipids of triglyceride-rich lipoproteins or in the triglycerides to the liver through elongation processes or are taken up directly by the mammary gland (Palmquist, 2009).

From omega-3 fatty acids, eicosapentaenoic acid (EPA, C20:5, omega-3) and docosahexaenoic acid (DHA, C22:6, omega-3) are the major dietary PUFAs and are mostly derived from fish oil (FO) (Calder, 2006). These long-chain fatty acids play an important role in immune and reproductive functions because they are precursors of the synthesis of different types of eicosanoids (Grez et al., 2016). Arachidonic acid (ARA, C20:4, omega-6), a component of omega-6 PUFAs, plays a central role in controlling the oestrous cycle through its derivative, prostaglandin F2α (PGF2α), which has luteolytic properties in farm animals (Verma et al., 2018). PUFAs, such as EPA, DHA, and ARA, have dual purposes: First, they are susceptible to reactive oxygen species (ROS)-induced peroxidative damage. Second, they can scavenge ROS, thereby reducing oxidative stress (Richard et al., 2008).

PUFAs play an important role in the biosynthesis of hormones involved in reproductive processes (Stillwell & Wassall, 2003). In addition to PGF2α, PUFAs affect the expression of genes involved in regulating lipid metabolism and function in progesterone production, which is the most important foetal protective hormone in the body (Jump, 2008). According to Wathes, Abayasekara, & Aitken (2007), PUFAs affect reproductive biological performance by affecting the development of follicles and the expression of genes encoding the enzymes required for the formation of prostaglandins and steroids. For example, Leroy, Van Soom, Opsomer, Goovaerts, & Bols (2008) fed dairy cows a diet supplemented with omega-3 fatty acids, which reduced the formation of PGF2α derived from ARA in the endometrium, increasing the viability of the corpus luteum (CL), and consequently embryo survival. This effect explains that EPA competes with ARA for the prostaglandin H synthetase enzyme binding sites responsible for ARA–prostaglandin conversion, and DHA directly inhibits the prostaglandin H synthetase activity (Roszkos, Tóth, & Mézes, 2020; Vedin et al., 2010).

Our objective in this study was to investigate the effects of omega-6 and omega-3 PUFA supplementation on the antioxidant status and sex hormone levels of modern genotype sows during lactation, breeding, and early gestation periods using sunflower oil (SO), a source of omega-6 fatty acids, as control and FO, a source of omega-3 fatty acids. Elevated oxidative stress is associated with oxidative deoxyribonucleic acid (DNA) damage during gestation and lactation in highly prolific multiparous sows (Berchieri-Ronchi et al., 2011). DNA damage was 47% throughout the lactational period and 21% during early gestation. Under oxidative stress, redox-sensitive glutathione (GSH) levels increase, which is followed by an increase in glutathione peroxidase (GPx) activity, which oxidizes GSH to glutathione disulphide by an oxygen free radical containing substrate (Bajic et al., 2019). Malondialdehyde (MDA) forms during the oxidation of unsaturated fatty acids and is a marker of the termination phase of lipid peroxidation (Morales & Munné-Bosch, 2019).

In this study, we examined 17β oestradiol (E2), progesterone (P4), and 6-keto prostaglandin F1α (6-keto-PGF1α) as markers of reproductive function. E2 is a major follicular hormone that plays a crucial role in follicle growth and development. After ovulation, P4, produced by the CL, is the most important hormone responsible for maintaining pregnancy. PGF2α quickly breaks down and is difficult to detect, so we measured 6-keto PGF1α, a stable metabolite. PGF2α controls the smooth muscle contractions of reproductive organs and causes luteolysis (CL regression) at the end of the oestrus cycle when fertilisation does not occur. Therefore, low P4 and high PGF2α levels during early pregnancy can cause early embryonic death (Geisert, Sutovsky, Lucy, Bartol, & Meyer, 2020).

Information is limited in the scientific literature on the effects of omega-3 fatty acids on the antioxidant system and reproductive hormones of swine (Chartrand et al., 2003; Clément et al., 1994; Frankic & Salobir, 2011; Habeanu et al., 2011; Lee & Kang, 2019; Lee et al., 2016; Smit et al., 2012). To our knowledge, researchers have not yet studied these effects on modern genotypes, such as Danish Large white × Danish Landrace. Nevertheless, a large amount of information is available for other animals, including ruminants and laboratory murine (Childs et al., 2008; Dirandeh et al., 2013; Leroy et al., 2008; Mahla et al., 2017; Robinson, Cheng, Wathes, & Abayasekara, 1998; Staples, Burke, & Thatcher, 1998; Vedin et al., 2010; Venkatraman, Chandrasekar, Kim, & Fernandes, 1994; Verma et al., 2018; Miralles-Pérez et al., 2021).

Researchers have found that feeding long-chain PUFAs to sows affects the fatty acid profile of their milk (Roszkos, George, Tóth, Fébel, & Mézes, 2021). The reduction in the omega-6/omega-3 ratio in milk showed that dietary omega-3 fatty acids could be successfully transferred to the milk and then advantageously used in the bodies of piglets for physiological purposes.

2 | MATERIALS AND METHODS

2.1 | Animals and experimental conditions

We conducted a trial with two replicates at a Hungarian swine farm working with Danish Large white × Danish Landrace as a breeding sow line terminated by Danish Duroc boars. We randomly divided 48 sows into 2 groups (24 each) in farrowing rooms. We selected sows for

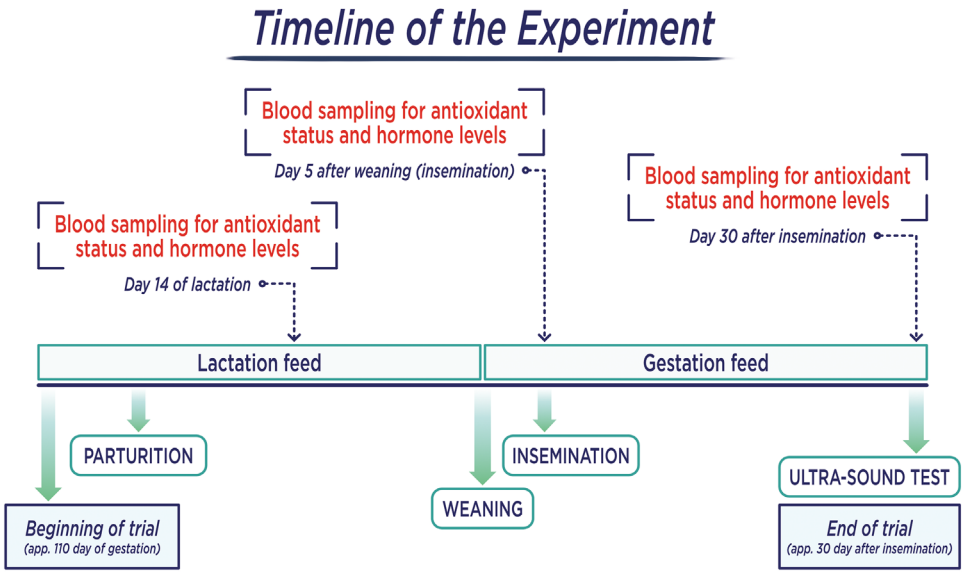

FIGURE 1 Timeline of the experiment

the control and experimental groups using the following parameters: between the second and fourth lactation, with at least 15 live-born piglets in the current and recent farrows.

We maintained control and experimental animals under the same conditions throughout the experiment. We started supplementation with control and experimental feeds 4–5 days before the expected parturition and ended with ultrasound tests on the 30th day of gestation (Figure 1). Starting on the first day of the experiment, we provided lactation feed to the animals until weaning. Between weaning and ultrasound testing, the animals ingested gestational feed. We fed sows according to the given feeding curves (Big Dutchman feeders; Big Dutchman International GmbH, Germany) during all experiments, and the sows had free access to drinking water.

During the experiment, we recorded the reproduction parameters (wean-to-oestrus interval, rate of late oestrus, pregnancy rate, and farrowing rate) of the sows.

2.2 | Dietary treatments

The lactation and gestation diets were based on corn–barley–soybean meal. We supplemented the experimental feed with an FO-based supplementary feed (FO on an inorganic carrier) at a rate of 10 g/kg feed. Simultaneously, the control group received SO-based supplementary feed (SO on an inorganic carrier) at the same rate (Table 1). The control and experimental supplements contained 63% of the original oil sources (SO or FO) and 37% of the inorganic carrier (silicon dioxide). FO and SO have almost the same energy content; therefore, we considered the diets isocaloric. We used oil-based supplementation instead of barley supplementation.

In the gestation phase, we did not mix the control and experimental supplementary feeds into the gestation diet; instead, we added them on top of the feed. As a result, the amount of the control and experimental supplementary feeds was 22.5 g per day, which was the same rate as during lactation.

Table 2 shows the omega-6 and omega-3 fatty acid contents of the supplementary feed we used. The omega-6 to omega-3 fatty acid ratio notably differed (1433 vs. 0.22) between the control and experimental supplementary feeds.

We could only examine the fatty acid content of the lactation feed, as we mixed control and experimental supplements into the lactation diets but not into the gestation diets (Table 3). The omega-6 to omega-3 fatty acid ratio between the control and experimental diets differed considerably (15.07 vs. 7.30).

2.3 | Chemical and other analyses

2.3.1 | Feed samples

We determined the fatty acid composition of the feed samples using the following method: We hydrolysed 1 g of feed sample with the addition of 40 mL of methanol, 6 mL of NaOH 50% solution, and 1.6 mL of the internal standard (500 mg of nonadecaenoic acid/100 mL toluene and isopropanol 2:1) at 80°C for 60 min. After chilling, we extracted lipids with chloroform, which evaporated under a nitrogen stream. We prepared fatty acid methyl esters with a 14% boron trifluoride/methanol solution and added 4 mL of hexane. Then, we vortexed the tube and stored approximately 1 mL of the upper phase containing the methyl esters in an amber-coloured glass bottle at −65°C for later analyses. We analysed fatty acid methyl esters using gas chromatography (gas chromatography–mass spectrometry; GCMS-QP2010 SE, Shimadzu, Kyoto, Japan). We separated the methyl ester components in a BPX70 capillary column (30 m × 0.25 mm × 0.25 μm, Phenomenex, USA). We performed GCMS analysis under the following conditions: injection temperature, 220°C; injection mode, split; flow control mode,

TABLE 1 Composition and calculated nutrient content of the sows fed control and experimental diets

	Lactation feeds		Gestation feeds	
Composition	Control diet	Experimental diet	Control diet	Experimental diet
Corn (%)	44.0	44.0	22.5	22.5
Barley (%)	26.7	26.7	51.3	51.3
Soybean meal (%)	15.0	15.0	0.50	0.50
Pressed sugar beet pulp (%)	–	–	12.0	12.0
Sunflower meal (%)	–	–	0.50	0.50
Alphasoy pig 530 (%)[a]	0.30	0.30	–	–
Dried apple (%)	0.20	0.20	–	–
Fish meal (%)	0.10	0.10	–	–
Vitamin and mineral premix (%)[b,c]	0.53	0.53	0.37	0.37
Animal fat supplement (%)[d]	0.20	0.20	0.05	0.05
Fish-oil-based supplementary feed[e] (%)	–	0.10	–	'on top'[f]
Sunflower-oil-based supplementary feed[e] (%)	0.10	–	'on top'[f]	–
Total (%)	100	100	100	100
Nutrient/energy content (calculated values, as in feed)				
Dry matter (%)	89.25	89.26	89.87	89.87
Digestible energy (DE_s, MJ/kg)	13.91	13.92	12.39	12.40
Metabolisable energy (ME_s, MJ/kg)	13.44	13.44	11.97	11.97
Crude protein (%)	16.03	16.03	12.75	12.75
Ether extract (%)	6.41	6.42	4.26	4.27
Crude fibre (%)	3.65	3.65	6.19	6.19
Ash (%)	5.46	5.45	4.87	4.86
SID Lys[g] (%)	0.83	0.83	0.55	0.55
SID Met[g] (%)	0.28	0.28	0.23	0.23
SID Met+Cys[g] (%)	0.51	0.51	0.43	0.43
SID Thr[g] (%)	0.54	0.54	0.41	0.41

[a]Agila (Denmark).
[b]Supplied per kilogram of lactation feed: vitamin A, 15 900 IU; vitamin D, 1 590 IU; vitamin E, 90 mg; vitamin K, 2.1 mg; thiamine, 1.6 mg; riboflavin, 5.3 mg; niacin, 26.5 mg; pantothenic acid, 18.6 mg; pyridoxine, 3.7 mg; folic acid, 0.8 mg; vitamin B12, 0.03 mg; I, 1.6 mg; Se, 0.42 mg; choline, 477 mg from choline chloride; Zn, 127.2 mg, Fe 95.4 mg, Mn 53 mg, and Cu 21.2 mg (Unimix Dan Lactation, Denmark).
[c]Supplied per kilogram of gestation feed: vitamin A, 11 100 IU; vitamin D, 1 110 IU; vitamin E, 59.2 mg; vitamin K, 1.5 mg; thiamine, 1.1 mg; riboflavin, 3.7 mg; niacin, 18.5 mg; pantothenic acid, 13 mg; pyridoxine, 2.6 mg; folic acid, 0.6 mg; vitamin B12, 0.02 mg; I, 1.1 mg; Se, 0.3 mg; choline, 333 mg from choline chloride; Zn, 88.8 mg, Fe 66.6 mg, Mn 37 mg, and Cu 14.8 mg (Unimix Dan Gestation, Denmark).
[d]ATEV (Hungary).
[e]Each feed supplement contained 6.3 g of FO or SO and 3.7 g silicon dioxide (SiO_2) per kilogram of supplement (Producer: ADEXGO Kft., Hungary).
[f]On top = 22.5 g per day added onto the feed.
[g]SID Lys = standardised ileal digestible lysine, SID Met = standardised ileal digestible methionine, SID Met+Cys = standardised ileal digestible methionine + cysteine, SID Thr = standardised ileal digestible threonine.

TABLE 2 Fatty acid content of oil supplementations used in the experiment

Analysed omega-6 and omega-3 fatty acid content (in supplement)	SO-based feed supplement (control)	FO-based feed supplement (experimental)
	mg fatty acid/g feed supplement	
C18:2 (omega-6)	303.02	23.75
C18:3 (omega-3)	0.21	8.80
C20:1 (omega-9)	0.99	34.32
C20:2 (omega-6)	–	1.61
C20:3 (omega-6)	–	0.61
C20:3 (omega-3)	–	0.78
C20:4 (omega-6)	–	2.28
C20:5 (omega-3)	–	47.28
C22:5 (omega-3)	–	14.26
C22:6 (omega-3)	–	57.89
Total omega-6 fatty acid	303.02	25.97
Total omega-3 fatty acid	0.21	96.31
Omega-6/omega-3	1433	0.22

Abbreviations: SO, sunflower oil; FO, fish oil.

linear velocity; pressure, 60.1 kPa; total flow, 45.4 mL/min; column flow, 1.03 mL/min; linear velocity: 37.1 cm/s; purge flow, 3 mL/min; split ratio, 40.

2.3.2 | Blood samples

The blood samples were collected three times: on day 14 of lactation (middle of lactation), 5 days after weaning (insemination), and on day 30 after insemination (early gestation, via ultrasound testing) into native (for hormone determinations) and heparinised (for biochemical parameters) vacuum tubes from 13 control and 13 experimental animals in the first replicate, and 11 control and 11 experimental sows in the second replicate (total $n = 48$).

The reduced GSH, GPx enzyme, and thiobarbituric acid reactive substances (TBARS) levels were determined in the blood plasma and red blood cell hemolysates. In addition, we determined the levels of E2, P4, and 6-keto-PGF1α in the blood serum.

Blood cells and plasma or serum was separated by centrifugation (2000× g for 15 min) and stored them at −70°C until analysis. Then, red blood cell hemolysates was made with nine-fold volume redistilled water, deep freezing (−70°C), and thawing (25°C).

The protein content of blood plasma and red blood cell hemolysates was determined using the biuret method, according to Weichselbaum (1946). The reduced GSH content of blood plasma and red blood hemolysates was determined based on the complex formation of nonprotein sulfhydryl groups with 5,5-dithiobis-(2-nitrobenzoic acid) following the method reported by Sedlak & Lindsay (1968). The GPx activity of blood plasma and red blood cell hemolysates was measured using an end-point direct assay with GSH and cumene hydroperoxide co-substrates (Matkovics, Szabó, & Varga, 1988). The TBARS content of blood plasma and red blood cell hemolysate was determined by the complex formation with 2-thiobarbituric acid, according to the method reported by Placer, Cushman, & Johnson (1966), and expressed as MDA which was served as standard.

The serum E2 and P4 levels were determined using a radioimmunoassay developed and validated by the Laboratory for Endocrinology, Department of Obstetrics and Reproduction (University of Veterinary Medicine, Hungary). For P4 level determination, the statistics were as follows: intra-assay coefficient of variation (CV), <5%; interassay CV, 10.2% (±3.4%); and analytical sensibility, 0.11 nmol/L. For E2 level determination, the conditions were as follows: intra-assay CV, <5%; interassay CV, 12.3%; analytical sensibility, 1.66 pg/mL.

The serum 6-keto-PGF1α level was measured using an enzyme-linked immunoassay developed and validated by the Laboratory for Endocrinology, Department of Obstetrics and Reproduction (University of Veterinary Medicine, Hungary). The statistics were as follows: intra-assay CV, <8%; interassay CV, 15.75% (±4.7%); analytical sensibility, 6 pg/mL.

2.4 | Statistical analysis

All statistical evaluations were performed using the SPSS 26.0 software package for Windows (IBM, Armonk, NY, USA). The antioxidant data were analysed using the general linear model procedure in SPSS. The statistical model included the effects of treatments (control and experimental diet), reproduction biology periods (day 14 of lactation, day 5 after weaning (insemination), and day 30 after insemination), replications, and their combinations (e.g., treatment × reproduction biology periods). We based our multiple comparisons of the observed means on Tukey's post hoc test.

The hormone data and reproduction biology parameters were analysed using the Kolmogorov–Smirnov test, the Levene test, and the independent-samples t-test or Mann–Whitney U test.

For all procedures, we set the significance level to $p \leq 0.05$. We further discussed differences at $0.05 < p \leq 0.1$ as tendencies.

3 | RESULTS

We evaluated the basic reproduction biology parameters in the experiment by comparing the combined control and experimental group data in the first and second replicates (Table 4). There were no significant differences between any of the parameters.; however, the weaning to oestrus interval (WOI) was one day shorter in the experimental group (4.35 days vs. 5.35 days). Additionally, the rate of animals in late oestrus in the experimental group was half that in the control group (3.85% vs. 7.69%).

The differences between the antioxidant and lipid peroxidation parameters (GSH, GPx, and TBARS) in the blood plasma and red blood

TABLE 3 Analysed fatty acid content of sows fed control and experimental diets

	Lactation feeds		Gestation feed
	Control (SO)	Experimental (FO)	Control/Experimental[a]
	mg fatty acid/g feed		
C18:2 (omega-6)	15.78	13.70	10.26
C18:3 (omega-3)	0.82	0.88	0.59
C20:1 (omega-9)	0.18	0.42	0.08
C20:2 (omega-6)	0.05	0.06	0.02
C20:3 (omega-6)	0.02	0.03	0.005
C20:3 (omega-3)	0.005	0.01	0.00
C20:4 (omega-6)	0.03	0.04	0.02
C20:5 (omega-3)	0.08	0.40	–
C22:5 (omega-3)	0.04	0.12	–
C22:6 (omega-3)	0.11	0.49	–
Omega-6/omega-3	15.07	7.30	17.43

Abbreviations: SO, sunflower oil; FO, fish oil.
[a]Normal gestation feed without SO or FO supplementation.

TABLE 4 Reproduction biology parameters of control and experimental sows during the trial ($n = 24$/treatment)

	CON (SO)	EXP (FO)	SEM	*p*-value
WOI (days)	5.35	4.35	0.430	0.249
Rate of late oestrus[a] (%)	7.69	3.85	–	–
Pregnancy rate (%)	100.00	100.00	–	–
Farrowing rate (%)	100.00	100.00	–	–

Abbreviations: CON, control diet; EXP, experimental diet; SO, sunflower oil; FO, fish oil; SEM, standard error of mean.
[a]Late oestrus = oestrus started more than 7 days after weaning.

cell hemolysates within the two replications (treatment × replication) were not statistically different. As such, we merged the values of the two replicates.

Compared with SO, FO feeding significantly changed the GPx activity in both the blood plasma and red blood cell hemolysates in the middle of lactation ($p = 0.009$ and $p = 0.003$, respectively) and the GSH level in red blood cell hemolysates 5 days after weaning (insemination) ($p = 0.038$) (Table 5).

The interaction between treatment and reproduction period also had a significant impact on the GPx levels in the blood plasma of FO-fed animals. The GSH content in red blood cell hemolysates of the SO group and three parameters (GSH, GPx, and TBARS) in the FO group were affected by this interaction, which showed that reproductive biology status affected these parameters ($p < 0.001$). During the statistical analysis of the hormone levels, we found no difference between the results of the replicates (treatment × replication); therefore, we combined the data.

Both E2 and 6-keto-PGF1α levels in the middle of lactation were significantly lower in the FO group ($p = 0.035$ and $p = 0.001$, respectively), but we found no difference in P4 levels (Table 6).

Plasma P4 levels were significantly higher ($p = 0.036$) in the FO group on the fifth day after weaning (insemination), whereas 6-keto-PGF1α levels tended to be lower ($p = 0.056$), and E2 levels did not differ ($p = 0.110$).

We found no significant difference in P4 levels during early gestation; however, E2 levels were significantly lower ($p = 0.012$) in the FO group, which was similar to those in the lactation period. In addition, 6-keto-PGF1α levels were also lower (tendency) in the FO group ($p = 0.077$).

4 | DISCUSSION

4.1 | Basic reproduction biology parameters

We found no significant difference between any of the basic reproductive biological parameters, including WOI, pregnancy, and farrowing rate. However, WOI was one day shorter in the experimental group (4.35 days vs. 5.35 days), which is an important difference from a practical point of view and may explain the alteration in P4 levels in the

TABLE 5 Antioxidant and lipid peroxidation values in blood plasma and blood cell hemolysate during the different reproduction biology periods of experiment ($n = 24$/treatment)

	Day 14 of lactation				Day 5 after weaning (insemination)				Day 30 after insemination (US testing)				*p*-Value (reproduction period × treatment)	
	CON (SO)	EXP (FO)	SEM	p	CON (SO)	EXP (FO)	SEM	P	CON (SO)	EXP (FO)	SEM	p	CON (SO)	EXP (FO)
Blood plasma														
GSH (mmol/g protein)	2.74	2.82	0.08	0.995	3.01	2.97	0.09	1.000	2.61	2.82	0.16	0.803	0.080	0.461
GPx (U/g protein)	2.87^{b}	3.28^{aA}	0.12	0.009	3.00	2.83^{B}	0.07	0.703	3.11	3.01^{B}	0.06	0.907	0.082	0.005
MDA (mmol/mL)	9.67	10.94	0.28	0.422	10.50	9.70	0.38	0.841	10.71	11.05	0.73	0.997	0.220	0.139
Red blood cell hemolysate														
GSH (mmol/ g protein)	5.93^{B}	5.04^{B}	0.21	0.082	7.38^{bA}	8.38^{aA}	0.24	0.038	5.26^{B}	5.30^{B}	0.26	1.000	<0.001	<0.001
GPx (U/g protein)	3.75^{b}	4.74^{aA}	0.17	0.003	3.89	3.75^{B}	0.16	0.995	4.08	3.79^{B}	0.22	0.910	0.505	<0.001
MDA (mmol/mL)	12.18	12.81^{AB}	0.37	0.947	13.17	14.17^{A}	0.49	0.722	13.09	11.21^{B}	0.64	0.132	0.344	<0.001

Note: a, b: Mean differences between the control and experimental groups within the same reproduction biology period at min. significance level of $p < 0.05$. A, B: Mean differences in the different reproduction biology periods within the control or experimental groups at the significance level of $p < 0.01$.

Abbreviations: CON, control diet; EXP, experimental diet; SO, sunflower oil; FO, fish oil; SEM, standard error of the mean; GSH, glutathione; GPx, glutathione peroxidase; MDA, malondialdehyde.

TABLE 6 Hormone values of blood samples in the different reproduction periods of the experiment ($n = 24$/treatment)

Period	Parameter	CON (SO)	EXP (FO)	SEM	*p*-Value
Day 14 of lactation	P4 (nmol/L)	4.99	4.85	0.12	0.561
	E2 (pg/mL)	4.60[a]	3.94[b]	0.15	0.035
	6-keto-PGF1α (pg/mL)	723[a]	201[b]	76.83	<0.001
Day 5 after weaning (insemination)	P4 (nmol/L)	4.94[b]	5.76[a]	0.19	0.036
	E2 (pg/mL)	5.72	5.31	0.12	0.110
	6-keto-PGF1α (pg/mL)	207	166[+]	12.67	0.056
Day 30 after insemination (US testing)	P4 (nmol/L)	52.58	49.95	1.12	0.301
	E2 (pg/mL)	5.84[a]	4.99[b]	0.17	0.012
	6-keto-PGF1α (pg/mL)	242	184[+]	17.08	0.077

Abbreviations: CON, control diet; EXP, experimental diet; SO, sunflower oil; FO, fish oil; SEM, standard error of mean; P4, progesterone; E2, 17β estradiol; 6-keto-PGF1α, 6-keto prostaglandin F1α.
[a,b]$p < 0.05$.
[+]Tendency: $0.05 < p < 0.1$.

blood. Our findings are similar to those obtained in previous studies. For instance, 0.5% salmon oil substitution for tallow in the lactation diet had no effect on the WOI of 48 Landrace × Yorkshire sows ($p > 0.05$) (Kibria, Choi, & Kim, 2021). The supplementation with 0.9% protected omega-3 fatty acids, fed starting 7 days before expected parturition until weaning, did not produce any differences ($p > 0.05$) in reproduction performance with corn–soybean–meal based standard diet (Wang, Yun, & Kim, 2021). Smits, Luxford, Mitchell, & Nottle (2011) fed 3.3 g/kg salmon oil as an experimental diet and a nonsupplemented control from day 107 of gestation until weaning to Large White × Landrace F1 sows and found no significant differences in WOI ($p = 0.130$), pregnancy ($p = 0.858$), or farrowing rate ($p = 0.797$) between the treatments.

Posser et al. (2018) found that microalgae (rich in DHA) supplementation, fed in different amounts (3.5, 7.0, 14.0, or 28.0 g/animal/day), did not affect the litter size or weight of PIC Camborough sows fed from 85 days of gestation through lactation to insemination. However, the WOI of sows supplemented with 7.0 g/day microalgae was longer (3.7 vs. 3.3; $p < 0.05$) than that of the control group.

Estienne, Harper, & Estienne (2006) observed no effect on WOI, ovulation rate, number, or weight of embryos after 68 days (41 prior to and 27 after farrow) of feeding 10 g/kg marine algae to 48 crossbred (Duroc × Yorkshire/Duroc × Yorkshire × Landrace) gilts after 18 days of progestin treatment. Contrary to the above, Rosero, Boyd, Odle, & van Heugten (2016) found that the dual supplementation of omega-6 and omega-3 fatty acids in different concentrations increased the number of sows coming to heat after weaning, reduced the WOI, enhanced the conception and farrowing rates, and decreased the culling rate of PIC Camborough sows in comparison with those of the nonsupplemented controls.

4.2 | Antioxidant system

In our study, the feeding of SO- or FO-based supplementary feeds with different omega-6/omega-3 fatty acid ratios did not cause oxidative stress in the blood plasma or red blood cell hemolysate of breeding sows, as shown by the TBARS content and the moderate change in the GSH redox system. However, the significant differences between the GPx activity on day 14 of lactation and day 5 after weaning indicated that FO affected the antioxidant defence. Additionally, the results showed that SO- or FO-based supplements fed at a dose of 10 g/kg did not adversely affect the antioxidant system of sows because they did not cause excessive GPx activity. As such, this dose can be considered safe in this respect.

Some researchers have examined the effect of supplementation with omega-3 fatty acids on the antioxidant and lipid peroxidation status of pigs (Frankic & Salobir, 2011; Habeanu et al., 2011; Lee & Kang, 2019) and laboratory rodents (Miralles-Pérez et al., 2021; Venkatraman et al., 1994), as well as in vitro studies with cell cultures (Lee et al., 2016; Sundaram, Giromini, Rebucci, & Baldi, 2020; Tatsumi et al., 2018). The main difference between the aforementioned studies and ours is that we used a positive control with a high amount of omega-6 fatty acids originating from the SO.

Our findings are in partial accordance with those of Lee & Kang (2019), who examined the cyclophosphamide (CTX)-induced oxidative challenge in 15 100-day-old male miniature pigs ([Duroc × Yorkshire] × [Pot Valley × Berkshire] × Yucatan) and fed a control diet or a diet rich in omega-3 fatty acids containing 55.75% ALA, 13.09% EPA, and 15.16% DHA. Their results showed that, in contrast with the control, the omega-3 treatment increased the activity of superoxide dismutase (SOD) and GPx levels and decreased the level of TBARS in the blood samples after the CTX challenge. The pronounced effects on the antioxidant system in that study were explained using CTX, which has powerful oxidative properties. However, our results contradict those of Frankic & Salobir (2011). They found that a 14-day supplementation with 9.5% linseed meal, containing 58% omega-3 PUFAs and 18% omega-6 PUFAs, increased the TBARS content in plasma.

In a previous study with laboratory animals, corn oil (CO), krill oil (KO), and FO were fed at a 10% inclusion rate to female mice, and the expression of different hepatic antioxidant enzymes' mRNA (e.g., SOD and GPx) was examined. Mice fed KO- and FO-based diets showed

significantly higher GPx and SOD enzyme activities ($p < 0.001$) in the liver cytosol compared with those fed the CO-based diet (Venkatraman et al., 1994). Omega-6 and omega-3 fatty acid feeding considerably affected the activity of antioxidant enzymes and, thus, the antioxidant status of the animals. Another laboratory animal study showed that a high dose (15%) of DHA-containing oil increased the TBARS levels in the blood plasma, liver, and kidney (Song, Fujimoto, & Miyazawa, 2000). Miralles-Pérez et al. (2021) found that feeding FO containing DHA in 80% of total fatty acids (0.8 mL oil/kg body weight intragastrically) promoted lipid peroxidation and protein carbonylation and activated the antioxidant response in rats.

Lee et al. (2016) examined the effects of ALA treatment during in vitro maturation (IVM) on nuclear maturation, intraoocyte GSH content, meiotic progression, and developmental competence after parthenogenesis and somatic cell nuclear transfer in pigs. Treatment with 100 μM ALA significantly increased ($p < 0.05$) intraoocyte GSH content (1.19 vs. 1.00 and 0.92 pixels per oocyte, comparing the treated oocytes, bovine serum albumin control, and porcine follicular fluid control, respectively). In addition, ALA treatment (100 μM) accelerated oocyte maturation, and a higher proportion of ALA-treated oocytes (89.6%) reached the MII stage than in the untreated controls (75.5%) at 33 h of IVM. In an in vitro study, Tatsumi et al. (2018) used immortalised mouse Schwann (IMS32) cells and examined the preventive effects of DHA and EPA (2.5–25 μM each) on oxidative-stress-induced cytotoxicity. The mRNA levels of several antioxidant enzymes (including GPx) were determined by real-time reverse transcription polymerase chain reaction. Both DHA and EPA levels increased the mRNA levels of several antioxidant enzymes in a dose-dependent manner, except those of SOD and GPx. However, DHA and EPA treatment substantially enhanced the intracellular GSH content compared to controls. Sundaram et al. (2020) used intestinal porcine epithelial cell line-J2 (IPEC-J2). They found that supplementation with EPA, DHA, and their 1:2 combination decreased oxidative damage in porcine epithelial cells induced by lipopolysaccharides, dextran sodium sulphate, or hydrogen peroxide.

4.3 | Hormones

According to our results, the feeding of FO-based supplementation substantially increased plasma progesterone (P4) levels on day 5 after weaning (insemination) compared with the controls fed SO-based supplementation. However, in other physiological phases, this feeding had no effect. The CL is responsible for the development of P4 production in the luteal phase after ovulation (Geisert et al., 2020). In our study, more animals ovulated earlier in the experimental group than in the control group, as the WOI was one day shorter (4.35 days vs. 5.35 days, a nonsignificant difference) in the experimental groups. Therefore, P4 production could have started earlier in the experimental group, which could explain their higher P4 plasma levels during fertilisation.

The increase in serum P4 levels following dietary omega-3 PUFA supplementation may be caused by reduced plasma clearance (Hawkins et al., 1995), decreased sensitivity of the CL to the luteolytic effects of PGF2α (Mattos, Staples, & Thatcher, 2000), or decreased production of PGF2α in the endometrium (MacLaren, Guzeloglu, Michel, & Thatcher, 2006).

Smit et al. (2012) fed PIC Camborough primiparous sows from 60 days of gestation to 21 days of lactation (weaning) with marine-oil-based, omega-3-rich supplementation at 84 g/animal/day, and the results were compared with those of a standard, control diet. The WOI, breeding, and pregnancy rates were similar among the examined groups. P4 concentrations in plasma 60 to 72 h after the calculated time of ovulation (9.94±0.62 mg/L for control and 9.17±0.64 mg/L for experimental) were not different ($p = 0.36$). Mitchell, Smits, Palmer, Filby, & Lane (2010) reported that 3 g/kg of FO supplementation for six weeks tended to increase the size of follicles compared with a standard commercial diet. In addition, significantly more embryos from sows fed FO diets developed to the blastocyst stage ($p < 0.05$) and tended to have a higher number of cells (p=0.06). Gene expression levels of E2, follicle-stimulating hormone receptors or prostaglandin receptors did not differ. Still, the expressions of P4 receptor and prostaglandin H synthetase in granulosa cells from FO-fed sows tended to be lower ($p < 0.07$).

The effect of feeding omega-3 PUFAs on P4 levels has mainly been investigated in ruminants. Verma et al. (2018) reported similar results with the supplementation of different FO doses in Rohilkhandi goats. Of the three different doses (72, 156, and 312 mg EPA and DHA/kg body weight), only the 156 mg dose significantly increased the P4 serum level on day 14 postoestrus compared with the levels in the control and other experimental groups. On the other hand, supplementation with 72 and 312 mg EPA and DHA/kg body weight substantially decreased serum P4 levels.

Robinson et al. (1998) and Dirandeh et al. (2013) observed a positive effect of dietary supplementation with an omega-3 PUFA-rich diet on P4 serum levels in cows. Robinson et al. (2002) found that a diet rich in ALA was associated with low plasma P4 concentrations in the early luteal phase, which may have been caused by reduced steroid hormone synthesis via decreased cholesterol levels as a precursor for P4 (Staples et al., 1998).

In this study, the blood levels of 17β-oestradiol (E2) were lower in the experimental group than in the control group in all three physiological phases (on day 14 of lactation and 30 days after insemination). These findings agree with those reported by Verma et al. (2018), where FO supplementation resulted in a significant decrease in serum E2 levels compared with the levels in the control group. In addition, our results are in agreement with those of previous studies on goats (Mahla et al., 2017) and dairy cows (Childs et al., 2008).

The reason for the lower E2 levels in the blood serum of experimental animals fed omega-3 PUFA could be the reduction in plasma cholesterol, which may have led to reduced steroid hormone synthesis, as cholesterol is also a precursor of E2 (Staples et al., 1998).

Compared with previous findings in swine, the E2 levels were quite low in our experiment (control: 4.60 ± 0.84 to 5.84 ± 0.80 pg/mL; experimental: 3.94 ± 0.72 to 5.31 ± 0.55 pg/mL). In a study by Edwards & Foxcroft (1983), the E2 levels before oestrus varied between 13 and 75 pg/mL (overall mean, 31 ± 4 pg/mL) in the plasma of multiparous

Landrace × Large White sows. Moreover, Almond & Dial (1990) found that serum E2 concentrations, excluding values of the preovulatory rise, ranged from 10 ± 1.7 to 16 ± 4.5 pg/mL in five crossbred primiparous sows. The genetic selection and evolution that have occurred in recent decades are possible reasons for the pronounced difference between the E2 levels in our study and those observed in previous studies.

The amount of 6-keto-PGF1α, indicating the PGF2α level, was significantly lower in the experimental group in all three periods (on day 14 of lactation) and two periods in a trend-like manner (on day 5 after weaning and on day 30 after insemination). According to these results, FO-based supplementation reduced the production of PGF2α in the experimental animals.

The omega-3 PUFAs found in FO, such as EPA and DHA, affect reproduction through the reduction in endometrial PGF2α secretion, which can be explained by the competitive inhibition of Δ6-desaturase, which prevents ARA synthesis and the suppression of prostaglandin H synthetase expression, which is responsible for ARA–PGF2α conversion (Thatcher & Staples, 2000).

Our results agree with those of Leroy et al. (2008), who found that the consumption of omega-3 PUFAs reduced the formation of PGF2α in the endometrium, which increased the vitality of the CL and, thus, the survival of embryos in dairy cows. Chartrand et al. (2003) also reported a reduction in PGF2α levels in plasma when linseed oil (ALA), as an omega-3 fatty acid source, was fed compared with feeding tallow during early pregnancy in Yorkshire-Landrace gilts. These authors concluded that the reduction in prostaglandin levels was responsible for the reduction in ARA or other eicosanoid substrates.

Clément et al. (1994) studied 21-day-old female large white piglets fed control or essential fatty acid (EFA)-deficient diets. The control diet contained 4000-mg LA and 300-mg ALA per 100 g of feed, whereas the EFA-deficient diet contained only 50-mg LA and 15-mg ALA per 100 g of feed. The researchers found that LA decreased from 26.2% ± 0.51% of total fatty acids in control to 5.6% ± 0.51% of total fatty acids in the EFA-deficient animals ($p < 0.0001$). The decrease in ARA was also notable: 7.46% ± 0.72% of total fatty acids in controls versus 4.75% ± 0.19% in the EFA-deficient animals ($p < 0.01$).

Other researchers studying murine macrophages have found that the increased ingestion of omega-3 PUFAs in the diet is associated with a decrease in the generation of active prostanoids such as two-series prostaglandins (Kelly et al., 1985). This may occur because omega-3 PUFAs replace ARA in the tissue phospholipids, which, when released, compete for prostaglandin synthase, thereby competitively attenuating the rate of two-series prostaglandin formation, which is derived from omega-6 PUFAs (Abayasekara & Wathes, 1999).

5 | CONCLUSIONS

In this study, the feeding of FO-based supplementary feed compared with SO-based supplementation at 10 g/kg substantially affected the GSH level and GPx activity in Danish Large white × Danish Landrace sows. On the other hand, omega-3 fatty acids at this dose facilitated the functioning of the antioxidant system but did not exert a pronounced antioxidant effect compared with the feeding of omega-6 PUFAs during the investigated physiological periods and did not adversely affect the antioxidant status of sows. Therefore, this dose is safe in this respect.

The omega-6 and -3 fatty-acid-induced changes in the investigated hormones (E2, P4, and 6-keto-PGF1α) have rarely been discussed in pigs; however, the presented results are similar to previous findings for other animal species, especially ruminants. This study provides evidence of the main impact of omega-6 and -3 fatty acids on the tested hormone levels, which serve as precursors for the production of sex hormones (E2 and P4) but have an opposite effect on PGF2α production. The omega-6 fatty acids favour PGF2α formation, whereas omega-3 fatty acids inhibit it, which we observed in all three investigated reproduction periods. The latter effect must be exploited during pig production, as reproduction is a key aspect in swine farms. From a practical perspective, our findings may have a considerable economic impact on intensive pig production.

Further investigations are needed to explore the economically relevant doses of omega-6 and omega-3 fatty acids, which can be successfully used in swine breeding and long-term trials, and to determine the complex effects of PUFAs on pig reproduction and health.

AUTHOR CONTRIBUTIONS

Conceptualisation, investigation, and writing—original draft, review, and editing: Róbert Roszkos. Conceptualisation, formal analysis, resources, supervision, validation, and writing—review and editing: Tamás Tóth. Formal analysis, supervision, and writing—review and editing: George Bazar. Methodology, supervision, and writing—review and editing: Hedvig Fébel. Conceptualisation, methodology, supervision, and writing—review and editing: Miklós Mézes.

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CONFLICT OF INTEREST

The authors state that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available on request from the corresponding author.

ETHICS STATEMENT

We conducted the experiment according to the Hungarian Animal Protection Act and relevant EU rules (Directive 2010/63/EU). The study was approved by the National Scientific Ethical Committee on Animal Experimentation, Hungary (approval number: PE/EA/872-8/2020).

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PEER REVIEW

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REFERENCES

Abayasekara, D. R. E., & Wathes, D. C. (1999). Effects of altering dietary fatty acid composition on prostaglandin synthesis and fertility. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *61*, 275–287. https://doi.org/10.1054/plef.1999.0101

Almond, G. W., & Dial, G. D. (1990). Steroid hormone and luteinizing hormone concentrations in the anestrous sow. *Canadian Journal of Veterinary Research*, *54*, 209–214.

Bajic, V. P., Van Neste, C., Obradovic, M., Zafirovic, S., Radak, D., Bajic, V. B., & Isenovic, E. R. (2019). Glutathione "redox homeostasis" and its relation to cardiovascular disease. *Oxidative Medicine and Cellular Longevity*, *2019*, 1–14. https://doi.org/10.1155/2019/5028181

Berchieri-Ronchi, C. B., Kim, S. W., Zhao, Y., Correa, C. R., Yeum, K. J., & Ferreira, A. L. A. (2011). Oxidative stress status of highly prolific sows during gestation and lactation. *Animal*, *5*, 1774–1779. https://doi.org/10.1017/s1751731111000772

Calder, P. C. (2006). N-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *American Journal of Clinical Nutrition*, *83*, 1505–1519. https://doi.org/10.1093/ajcn/83.6.1505S

Chartrand, R., Matte, J. J., Lessard, M., Chouinard, P. Y., Giguere, A., & Laforest, J. P. (2003). Effect of dietary fat sources on systematic and intrauterine synthesis on prostaglandins during early pregnancy of gilts. *Journal of Animal Science*, *81*, 726–784. https://doi.org/10.2527/2003.813726x

Childs, S., Lynch, C. O., Hennessy, A. A., Stanton, C., Wathes, D. C., Sreenan, J. M., & Kenny, D. A. (2008). 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*, *2*, 883–893. https://doi.org/10.1017/s1751731108002115

Clément, G., Christon, R., Créminon, C., Frobert, Y., Pradelles, P., & Wal, J. M. (1994). Essential fatty acid deficiency in the pig: Effects on eicosanoid basal levels and in vitro synthesis by the small intestine. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *50*, 147–154. https://doi.org/10.1016/0952-3278(94)90098-1

Dirandeh, E., Towhidi, A., Ansari, P. Z., Adib, H. F., Ganjkhanloua, M., Zeinoaldini, S., Rezaei, R. A., Saberifar, T., & Petit, H. V. (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*, 131–137. http://doi.org/10.1016/j.theriogenology.2013.03.012

Edwards, S., & Foxcroft, G. R. (1983). Endocrine changes in sows weaned at two stages of lactation. *Journal of Reproduction and Fertility*, *67*, 161–172. https://doi.org/10.1530/jrf.0.0670161

Estienne, M. J., Harper, A. F., & Estienne, C. E. (2006). Effects of dietary supplementation with omega-3 polyunsaturated fatty acids on some reproductive characteristics in gilts. *Reproduction Biology*, *6*, 231–241.

Frankic, T., & Salobir, J. (2011). In vivo antioxidant potential of sweet chestnut (*Castanea sativa* Mill.) wood extract in young growing pigs exposed to n-3 PUFA-induced oxidative stress. *Journal of the Science of Food and Agriculture*, *91*, 1432–1439. https://doi.org/10.1002/jsfa.4328

Geisert, R. D., Sutovsky, P., Lucy, M., Bartol, F. F., & Meyer, A. E. (2020). Reproductive physiology of swine. In: Bazer FW, Lamb GC, Wu G (Eds.), *Animal agriculture: Challenges, innovations, and sustainability*. (1st ed., pp. 263–282). Academic Press. https://doi.org/10.1016/b978-0-12-817052-6.00015-x

Grez, M., Gandarillas, M., González, F., & Vargas-Bello-Pérez, M. (2016). Influence of a spray-dried fat enriched with EPA and DHA on the fatty acid composition of sow milk. *Ciencia e Investigacion Agraria*, *43*, 347–355. https://doi.org/10.4067/S0718-16202016000300001

Habeanu, M., Hebean, V., Nagy, A., Taranu, I., Lefter, N. A., Marin, D., & Grosu, H. (2011). The dietary omega-3 PUFA alter the metabolic and immunologic serum profile in Mangalitza pigs in rearing extensive system. *Archives of Zootechnology*, *14*, 5–12.

Hawkins, D. E., Niswender, K. D., Oss, G. M., Moeller, C. L., Odde, K. G., Sawyer, H. R., & Niswender, G. D. (1995). An increase in serum lipids increases luteal lipid content and alters disappearance rate of progesterone in cows. *Journal of Animal Science*, *73*, 541–545. https://doi.org/10.2527/1995.732541x

Jump, D. B. (2008). N-3 polyunsaturated fatty acid regulation of hepatic gene transcription. *Current Opinion on Lipidology*, *19*, 242–247. https://doi.org/10.1097/MOL.0b013e3282ffaf6a

Kelly, V. E., Ferretti, A., Izui, S., & Strom, T. B. (1985). A fish oil diet rich in eicosapentaenoic acid reduces cyclooxygenase metabolites and suppresses lupus in MRL-lpr mice. *Journal of Immunology*, *134*, 1914–1919.

Kibria, S., Choi, Y. J., & Kim, I. H. (2021). Effect of omega-3 fatty acid supplementation in salmon oil on the production performance of lactating sows and their offspring. *Korean Journal of Agricultural Science*, *48*, 191–199. https://doi.org/10.7744/kjoas.20210002

Lee, Y., Lee, H., Park, B., Elahi, F., Lee, J., Lee, S. T., & Lee, E. (2016). Alpha-linolenic acid treatment during oocyte maturation enhances embryonic development by influencing mitogen-activated protein kinase activity and intraoocyte glutathione content in pigs. *Journal of Animal Science*, *94*, 3255–3263. https://doi.org/10.2527/jas.2016-0384

Lee, S. I., & Kang, K. S. (2019). Omega-3 fatty acids modulate cyclophosphamide induced markers of immunosuppression and oxidative stress in pigs. *Scientific Reports*, *9*, 2684. https://doi.org/10.1038/s41598-019-39458-x

Leroy, J. L., Van Soom, A., Opsomer, G., Goovaerts, I. G., & Bols, P. E. (2008). Reduced fertility in high-yielding dairy cows: Are the oocyte and embryo in danger? Part II. *Reproduction of Domestic Animals*, *43*, 623–632. https://doi.org/10.1111/j.1439-0531.2007.00961.x

MacLaren, L. A., Guzeloglu, A., Michel, F., & Thatcher, W. W. (2006). Peroxisome proliferator-activated receptor (PPAR) expression in cultured bovine endometrial cells and response to omega-3 fatty acid, growth hormone and agonist stimulation in relation to series 2 prostaglandin production. *Domestic Animal Endocrinology*, *30*, 155–169. https://doi.org/10.1016/j.domaniend.2005.07.0

Mahla, A. S., Chaudhari, R. K., Verma, A. K., Singh, A. K., Singh, S. K., Singh, G., & Krishnaswamy, N. (2017). Effect of dietary supplementation of omega-3 polyunsaturated fatty acid (PUFA) rich fish oil on reproductive performance of the goat (*Capra hircus*). *Theriogenology*, *99*, 79–89. https://doi.org/10.1016/j.theriogenology.2017

Matkovics, B., Szabó, L., & Varga, I. (1988). Determination of enzyme activities in lipid peroxidation and glutathione pathways (In Hungarian). *Laboratóriumi Diagnosztika*, *15*, 248–250.

Mattos, R., Staples, C. R., & Thatcher, W. W. (2000). Effects of dietary fatty acids on reproduction in ruminants. *Reviews of Reproduction*, *5*, 38–45. https://doi.org/10.1530/ror.0.0050038

Miralles-Pérez, B., Méndez, L., Nogués, M. R., Sánchez-Martos, V., Fortuño-Mar, A., Ramos-Romero, S., Hereu, M., Medina, I., & Romeu, M. (2021). Effects of a fish oil rich in docosahexaenoic acid on cardiometabolic risk factors and oxidative stress in healthy rats. *Marine Drugs*, *19*, 555. https://doi.org/10.3390/md19100555

Mitchell, M., Smits, R., Palmer, N. O., Filby, A. N., & Lane, M. (2010). Dietary omega-3 fatty acid supplementation alters embryo development and metabolism in sows. *Reproduction Fertility and Development*, *22*, 137–137. https://doi.org/10.1071/SRB10Abs337

Morales, M., & Munné-Bosch, S. (2019). Malondialdehyde: Facts and artifacts. *Plant Physiology*, *180*, 1246–1250. https://doi.org/10.1104/pp.19.00405

Palmquist, D. L. (2009). Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. *The Professional Animal Scientist*, *25*, 207–249. https://doi.org/10.15232/S1080-7446(15)30713-0

Placer, Z. A., Cushman, L. L., & Johnson, B. C. (1966). Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Analytical Biochemistry*, *16*, 359–364. https://doi.org/10.1016/0003-2697(66)90167-9

Posser, C. J. M., Almeida, L. M., Moreira, F., Bianchi, I., Gasperin, B. G., & Lucia, T. (2018). Supplementation of diets with omega-3 fatty acids from microalgae: Effects on sow reproductive performance and metabolic parameters. *Livestock Science*, *207*, 59–62. https://doi.org/10.1016/j.livsci.2017.11.006

Richard, D., Kefi, K., Barbe, U., Bausero, P., & Visioli, F. (2008). Polyunsaturated fatty acids as antioxidants. *Pharmacology Research*, *57*, 451–455. https://doi.org/10.1016/j.phrs.2008.05.002

Robinson, R. S., Cheng, Z., Wathes, D. C., & Abayasekara, D. R. E. (1998). Effect of dietary polyunsaturated fatty acids (PUFAs) on bovine luteal cell steroidogenesis in vitro. *Journal of Endocrinology*, *159*, 67.

Robinson, R. S., Pushpakumara, P. G. A., Cheng, Z., Peters, A. R., Abayasekara, D. R. E., & Wathes, D. C. (2002). Effects of dietary polyunsaturated fatty acids on ovarian and uterine function in lactating dairy cows. *Reproduction*, *124*, 119–131. https://doi.org/10.1530/rep.0.1240119

Rosero, D. S., Boyd, R. D., Odle, J., & van Heugten, E. (2016). Optimizing dietary lipid use to improve essential fatty acid status and reproductive performance of the modern lactating sow: A review. *Journal of Animal Science and Biotechnology*, *7*, 34. https://doi.org/10.1186/s40104-016-0092-x

Roszkos, R., Tóth, T., & Mézes, M. (2020). Review: Practical use of n-3 fatty acids to improve reproduction parameters in the context of modern sow nutrition. *Animals*, *10*, 1141, https://doi.org/10.3390/ani10071141

Roszkos, R., George, B., Tóth, T., Fébel, H., & Mézes, M. (2021). Effect of n-3 polyunsaturated fatty acid feeding on the fatty acid profile and odor of milk in danbred sows. *Journal of Applied Animal Research*, *49*, 447–459. https://doi.org/10.1080/09712119.2021.2005071

Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*, *25*, 192–205. https://doi.org/10.1016/0003-2697(68)90092-4

Simopoulos, A. P. (2002). Omega-3 fatty acids in infammation and autoimmune diseases. *Journal of the American College of Nutrition*, *21*, 495–505. https://doi.org/10.1080/07315724.2002.10719248

Smit, M. N., Patterson, J. L., Webel, S. K., Spencer, J. D., Cameron, A. C., Dyck, M. K., & Foxcroft, G. R. (2012). Responses to n-3 fatty acid (LCPUFA) supplementation of gestating gilts, and lactating and weaned sows. *Animal*, *7*, 784–792. https://doi.org/10.1017/s1751731112002236

Smits, R. J., Luxford, B. G., Mitchell, M., & Nottle, M. B. (2011). Sow litter size is increased in the subsequent parity when lactating sows are fed diets containing n-3 fatty acids from fish oil. *Journal of Animal Science*, *89*, 2731–2738. https://doi.org/10.2527/jas.2010-3593

Sprecher, H. (2000). Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochimica et Biophysica Acta*, *1486*, 219–231. https://doi.org/10.1016/S1388-1981(00)00077-9

Staples, C. R., Burke, J. M., & Thatcher, W. W. (1998). Influence of supplemental fats on reproductive tissues and performance of lactating cows. *Journal of Dairy Science*, *81*, 856–871. https://doi.org/10.3168/jds.s0022-0302(98)75644-9

Stillwell, W., & Wassall, S. R. (2003). Docosahexaenoic acid: Membrane properties of a unique fatty acid. *Chemistry and Physics of Lipids*, *126*, 1–27. https://doi.org/10.1016/s0009-3084(03)00101-4

Song, J. H., Fujimoto, K., & Miyazawa, T. (2000). Polyunsaturated (n-3) fatty acids susceptible to peroxidation are increased in plasma and tissue lipids of rats fed docosahexaenoic acid-containing oils. *The Journal of Nutrition*, *130*, 3028–3033. https://doi.org/10.1093/jn/130.12.3028

Sundaram, T. S., Giromini, C., Rebucci, R., & Baldi, A. (2020). Omega-3 polyunsaturated fatty acids counteract inflammatory and oxidative damage of non-transformed porcine enterocytes. *Animals*, *10*, 956. https://doi.org/10.3390/ani10060956

Tatsumi, Y., Kato, A., Sango, K., Himeno, T., Kondo, M., Kato, Y., & Kato, K. (2018). Omega-3 polyunsaturated fatty acids exert antioxidant effects through the Nrf2 pathway in the immortalized mouse schwann IMS32 cells. *Journal of Diabetes Investigation*, *10*, 602–612. https://doi.org/10.1111/jdi.12931

Thatcher, W. W., & Staples, C. R. (2000). Effects of dietary fat supplementation on reproduction in lactating dairy cows. *Advanced Dairy Technology*, *12*, 213–232.

Vedin, I., Cederholm, T., Freund-Levi, Y., Basun, H., Hjorth, E., Irwing, G. F., Jönhagen, M. E., Schultzberg, M., Wahlund, L. O., & Palmblad, J. (2010). Reduced prostaglandin F2 alpha release from blood mononuclear leukocytes after oral supplementation of omega3 fatty acids: The OmegAD study. *Journal of Lipid Research*, *51*, 1179–1185. https://doi.org/10.1194/jlr.M002667

Venkatraman, J. T., Chandrasekar, B., Kim, J. D., & Fernandes, G. (1994). Effects of n−3 and n−6 fatty acids on the activities and expression of hepatic antioxidant enzymes in autoimmune-prone NZB×NZW F1 mice. *Lipids*, *29*, 561–568. https://doi.org/10.1007/bf02536628

Verma, A. K., Mahla, A. S., Chaudhari, R. K., Singh, A. K., & Khatti, A. (2018). 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*). *Animal Reproduction Science*, *195*, 153–161. https://doi.org/10.1016/j.anireprosci.2018.05

Wang, H., Yun, M. H., & Kim, I. H. (2021). Evaluation of dietary-coated omega-3 fatty acid supplementation on reproduction performance, growth performance, nutrient digestibility, and blood profiles in lactating sows and suckling piglets. *Canadian Journal of Animal Science*, *101*, 473–480. https://doi.org/10.1139/cjas-2020-0074

Wathes, D. C., Abayasekara, D. R. E., & Aitken, R. J. (2007). Polyunsaturated fatty acids in male and female reproduction. *Biology of Reproduction*, *77*, 190–201. https://doi.org/10.1095/biolreprod.107.060558

Weichselbaum, T. E. (1946). An accurate and rapid method for the determination of protein in small amounts of blood, serum and plasma. *American Journal of Clinical Pathology*, *10*, 40–49.

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