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Fish oil rich in eicosapentaenoic acid and docosahexaenoic acid in sow diets modifies oxylipins and immune indicators in colostrum and milk

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ABSTRACT

Colostrum and milk are the first nutrient sources for newborn piglets. In addition, n-3 fatty acids (**FAs**) and their oxygenated derivatives (oxylipins) have the capacity to modulate immune components. The aim of the current study was to include a fish oil rich in eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) in sow diets to promote an increase of anti-inflammatory molecules in colostrum and milk to benefit piglets. Thirty-six sows were randomly assigned from insemination to the end of lactation to either a control diet with animal fat (15 g/kg in gestation and 30 g/kg in lactation) or an n-3 diet in which animal fat was totally (gestation) or half (lactation) replaced by an equivalent amount of solid fish oil. Performance of sows and piglets was monitored during the study. Colostrum and milk samples were obtained after the birth of the first piglet and at weaning, respectively. From all samples (n = 18 per treatment), FAs were quantified by gas chromatography and immunoglobulins and cytokines by ELISA. Three samples per treatment were randomly selected to analyse oxylipin composition by liquid chromatography-tandem mass spectrometry. In colostrum and in milk, the n-3 FA (P = 0.020 and P < 0.001), particularly EPA (P < 0.001 and P < 0.001) and DHA (P < 0.001 and P < 0.001), and also their oxygenated derivatives were increased in samples from sows fed n-3 diet. Fish oil had no effect on immunoglobulin concentrations, but reduced tumour necrosis factor α (**TNFα**) (P = 0.011) and a tendency to reduce interleukin 10 (**IL10**) (P = 0.059) were observed in milk. In conclusion, fish oil in sow diets increased n-3 FA, particularly EPA and DHA, and their oxygenated derivatives in colostrum and milk, reducing TNFα and IL10 in milk.

Implications

This study shows that the inclusion of fish oil in sow diets during gestation and lactation may reduce litter size without affecting total litter weight during lactation and increases the concentration of n-3 fatty acids and their oxygenated derivatives in colostrum and milk. Further studies with larger number of animals are needed to assess the effects of n-3 fatty acids on litter size and piglet's growth performance. On the other hand, the transfer of these anti-inflammatory molecules to suckling piglets could result in an improved immune status of newborn piglets.

Introduction

Colostrum and milk are the earliest nutrient sources for the newborn piglets. They contain a mixture of constituents such as lactose, proteins (mainly casein and immunoglobulins), fatty acids (**FAs**), growth factors and immune cells that are crucial for the transfer of energy and maternal passive immunity (Klobasa et al., 1987; Darragh and Moughan, 1998). However, they differ in time of secretion and in composition. Colostrum is the first secretion of the mammary glands, and it is produced within the first 24 h after farrowing. It is mainly composed of immunoglobulins (**Ig**) (concretely IgG) and it is the main energy source for piglets immediately after birth (Devillers et al., 2011). Its composition rapidly changes to that of mature milk as fat and lactose concentrations increase and protein and Ig decrease (Quesnel et al., 2015). Considering that piglets are born with low body fat and energy reserves,

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and devoid of immune protection (Le Dividich et al., 2005), colostrum and milk play a critical role for piglet survival.

Several studies grouped in the review of Rosero et al. (2016) have shown that lipid supplementation to lactation sow diets slightly reduce sow BW loss during lactation and increased fat content in milk resulting in a higher energy intake for piglets which could positively improve their growth. However, the role of individual or families of FA has only recently been evaluated (Rosero et al., 2016). For this reason, the nature of lipids used in sow diets needs to be examined in detail (Bontempo and Jiang, 2015). Different studies found that the different sources of dietary polyunsaturated FAs increase their concentration in milk (Smit et al., 2015; Gessner et al., 2016). It is well established that these polyunsaturated FAs have an impact on the immune status (Lauridsen, 2020) through the modification of the oxylipin profile (Balvers et al., 2012) and its subsequent effect on the synthesis of cytokines (Calder, 2010). Oxylipins are oxygenated lipid mediators and are the major mediators for polyunsaturated FA effects in the body (Gabbs et al., 2015). Because they are considered as bioactive lipid mediators critically involved in neonatal physiology (Wu et al., 2016), their presence in human milk (Robinson et al., 2017) suggests that they may have relevant effects on the immune status of the newborn after colostrum and milk intake. There are differences between n-6 and n-3 polyunsaturated FAs in their effects on immune status, as n-6 polyunsaturated FAs are precursors of proinflammatory oxylipins, while n-3 polyunsaturated FAs are precursors of anti-inflammatory oxylipins (Calder, 2010). To our knowledge, no prior studies have analysed oxylipin concentration in colostrum in any species and there are only some studies in bovine (Kuhn et al., 2017) and human (Robinson et al., 2017) milk, but not in sows.

In the current study, it is hypothesised that fish oil in sow diets promotes the increase of n-3 polyunsaturated FA and n-3 polyunsaturated FA-derived oxylipins in colostrum and milk, which could have an impact on their immunological properties. Therefore, the aim was to evaluate the impact of a solid fish oil rich in eicosapentaenoic acid (**EPA**) (C20:5 n-3) and docosahexaenoic acid (**DHA**) (C22:6 n-3) in sow diets on the nutritional composition of colostrum and milk (FA content, oxylipins, Ig and cytokines) which will be transferred to the newborn piglet and could affect their growth and immune status. Preliminary results have been published in an abstract form (Llauradó-Calero et al., 2020).

Material and methods

Animals, housing and experimental design

The study was performed with thirty-six sows in four consecutive batches (12, 9, 5 and 10 sows in the 1st, 2nd, 3rd and 4th batch, respectively). The different number of sows differs between batches due to the availability of sows that met the selection criteria of being between the 3rd parity and 6th parity. In each batch, sows were divided into groups of two as similar as possible regarding parity number and BW. Within each group, sows were randomly assigned to a control diet or an n-3 long-chain FAs (**LCFA**) rich diet. Sows were involved in the trial from insemination until the end of lactation (±28 days after farrowing). Cross fostering of piglets was only performed during the first 24 h postfarrowing to standardise litter size (12 piglets per sow), and exclusively among sows belonging to the same experimental treatment. At day eleven of lactation, a control or an n-3 LCFA prestarter creep feed for piglets was introduced in accordance with the maternal diet.

Sows were allocated to individual stalls from insemination to pregnancy confirmation, afterwards till one week before farrowing sows were group-housed in a gestation barn and one week before farrowing, they were moved to individual farrowing crates (0.7 × 2 m) equipped with partially slatted floor and a heated floor panel for piglets (set at 32–34 °C). The room was lit via skylight and artificial light (non-programmable), and its ventilation was via single, variable-speed fans linked to temperature sensors. The temperature inside the building was automatically controlled. The target temperature of the rooms was set at 24 °C at farrowing, and it was reduced by 0.5 °C per week until weaning. Sows were fed via hoppers and piglets from round feeders on the ground. Water was provided *ad libitum* from nipple drinkers.

Experimental diets

Gestation and lactation diets were formulated according to FEDNA specifications (de Blas et al., 2013). Sows were fed either a control diet with animal fat (15 and 30 g/kg for gestation and lactation specifications, respectively) or an n-3 LCFA diet in which animal fat was totally (gestation) or half (lactation) replaced by an equivalent amount of a solid fish oil (Lipomega®; V&S Asociados, Madrid, Spain). For piglets, the control prestarter creep feed was formulated to contain 30 g/kg of animal fat and in the n-3 LCFA diet, it was totally replaced by an equivalent amount of solid fish oil. The diets were formulated to contain the same level of the main nutrients (metabolisable energy, CP, digestive lysine, and ether extract) (Table 1) except for the FA composition (Table 2). Feed intake was restricted to a maximum of 3 kg/day during gestation, and gradually increased after farrowing until reaching *ad libitum* feed intake.

Growth measurements and sampling

Sows were weighed at insemination, at entering the farrowing unit (day 107 of gestation), the day after farrowing and at weaning. Daily feed intake was monitored individually throughout the study and recorded for each period (gestation and lactation). Average daily gain during gestation was calculated from insemination to day 107 of gestation, and average daily gain during lactation from the day after farrowing to weaning. The number of piglets at birth, piglets born alive/death, mummies and their individual weight were monitored at birth. For lactation, piglets were weighted at 24 h, 20 days after birth and at weaning. All adoptions were completed within 24 h after birth, and the 24 h recordings were considered as the initial values for the litter characteristics and growth performance of suckling piglets. Thus, litter and piglet's average daily gain were calculated between 24 h and 20 days after birth or weaning. Creep feed disappearance was monitored from day eleven after birth until weaning.

Colostrum samples from each sow were obtained immediately after birth of the first piglet, and milk samples were collected at the end of lactation after the piglet's removal. Sows were milked from all mammary glands after i.v. injection of 1.0 mL of oxytocin (20 IU/mL) (Super's Diana S.L., Parets del Vallès, Spain). Samples from different nipples in each sow were pooled and aliquots for FA, oxylipins (with tubs containing 0.005% butylated hydroxytoluene (Merck, Darmstadt, Germany) as antioxidant), Ig and cytokines were stored at −80 °C until analysis.

One sow offered n-3 LCFA diet farrowed out of the scheduled time, without supervision, and litter characteristics at birth and colostrum sampling were not possible. Moreover, two sows from the same n-3 LCFA group gave birth to less than six piglets and were excluded for the analysis of litter characteristics at birth.

Quantitative analysis of fatty acids

Fat was extracted from all colostrum and milk samples with chloroform (PanReac AppliChem, Barcelona, Spain) - methanol

Table 1
Ingredient and nutrient composition of the gestation and lactation sow diets and piglets creep feed (as fed basis).

	Gestation		Lactation		Creep feed	
	Control	n-3 LCFA	Control	n-3 LCFA	Control	n-3 LCFA
Ingredient (g/kg)						
Barley	443	435	–	–	226	220
Corn	200	200	508	499	314	315
Sunflower seed 37%	100	100	40.0	40.0	–	–
Soybean hulls	–	–	82.3	84.3	–	–
Wheat middlings	80.0	80.0	–	–	–	–
Sugar-beet pulp	80.0	80.0	60.0	60.0	–	–
Soybean 48%	40.6	42.7	240	241	150	150
Whey, sweet, skim (dehydrated)	–	–	–	–	110	110
Dicalcium phosphate	15.4	15.4	19.2	19.3	18.2	18.2
Animal fat (5 Sysfeed)[1]	15.0	–	30.0	15.0	30.0	–
Fish oil (Lipomega®)[2]	–	21.5	–	21.5	–	48.6
L-lysine HCL	1.30	1.30	1.37	1.34	5.50	5.50
L-threonine	0.20	0.20	0.46	0.45	2.50	2.50
DL-methionine	–	–	0.24	0.24	2.70	2.70
L-tryptophan	–	–	0.17	0.17	0.80	0.80
L-Valine	–	–	–	–	1.30	1.30
Calcium carbonate	10.5	10.4	6.92	6.83	2.00	2.70
Sodium bicarbonate	8.50	8.50	–	–	4.50	4.50
Sodium chloride	0.80	0.80	4.63	4.62	0.80	0.80
Sodium caseinate	–	–	–	–	20.0	20.0
Celite	–	–	–	–	15.0	–
HP 300[3]	–	–	–	–	88.8	89.3
Vitamin-Mineral premix[4,5]	4.00	4.00	4.00	4.00	6.00	6.00
Antioxidant (Noxyfeed 56P)[6]	0.30	0.30	2.50	2.50	2.50	2.50
Analysed nutrient composition[7] (g/kg)						
ME (MJ/kg)	12.4	12.5	13.7	13.6	14.1	13.8
DM	903	905	887	888	875	876
Crude fibre	57.9	60.4	54.3	54.5	19.0	18.7
Ether extract	38.6	37.2	56.7	59.2	49.8	52.4
CP	131	132	182	179	204	205
Lysine	56.0	56.0	92.0	92.0	13.3	13.3

LCFA, long-chain fatty acid; ME, metabolisable energy.
[1] Product of Sysfeed SLU (Granollers, Spain). It contains myristic acid (C14:0) 1.50%, palmitic acid (C16:0) 18.0%, palmitoleic acid (C16:1 n-7) 2.00%, stearic acid (C18:0) 14.0%, oleic acid (C18:1 n-9 *cis*) 28.0%, linoleic acid (C18:2 n-6 *cis*) 12.0%, α-linolenic acid (C18:3 n-3 *cis*) 6.00%, saturated–unsaturated 0.7%.
[2] Product of V&S Asociados (Madrid, Spain). It contains 63.36% of fat, myristic acid (C14:0) 4.79%, palmitic acid (C16:0) 14.9%, stearic acid (C18:0) 3.77%, oleic acid (C18:1 n-9 *cis*) 12.3%, linoleic acid (C18:2 n-6 *cis*) 2.71%, α-linolenic acid (C18:3 n-3 *cis*) 1.21%, arachidonic acid (C20:4 n-6 *cis*) 0.75%, eicosapentaenoic acid (C20:5 n-3 *cis*) 7.92%, docosahexaenoic acid (C22:6 n-3 *cis*) 6.91% and 36.64% of the inert excipient Tixosil® silica (Solvay, Brussels, Belgium).
[3] Product of Hamlet Protein (Horsens, Denmark). It contains 56.0% of protein, 23.2% of carbohydrates, 8.0% of H_2O, 6.8% of ash, 3.5% of crude fibre and 2.5% of fat. Essential amino acids (g/16 g of N): lysine 6.1, methionine 1.3, cysteine 1.4, threonine 3.9, tryptophan 1.35, leucine 7.7, isoleucine 4.6, phenylalanine 5.0, tyrosine 3.7, valine 4.8, histidine 2.6 and arginine 7.2.
[4] Vitamin-Mineral premix (sows): Product of TecnoVit S.L. (Alforja, Spain). Supplied per kilogram of feed: vitamin A (E-672) 10,000 UI; vitamin D_3 (E-671) 1 600 UI; vitamin E (alfa-tocopherol) 15 mg; vitamin B_1 1 mg; vitamin B_2 2.7 mg; vitamin B_6 1.8 mg; vitamin B_{12} 15 μg; vitamin K_3 1 mg; calcium panthotenate 11 mg; nicotinic acid 15 mg; folic acid 1 mg; biotin 100 μg; choline 200 mg; Fe (E-1) (from $FeSO_4{\cdot}7H_2O$) 150 mg; I (E-2) (from $Ca(IO3)_2$) 0.5 mg; Co (E-3) (from $2CoCO_3{\cdot}3Co(OH)_2{\cdot}H_2O$) 0.5 mg; Cu (E-4) (from $CuSO_4{\cdot}5H_2O$) 10 mg; Mn (E-5) (from MnO) 40 mg; Zn (E-6) (from ZnO) 100 mg; Se (E-8) (from Na_2SeO_3) 0.25 mg.
[5] Vitamin-Mineral premix (piglets): Product of TecnoVit S.L. (Alforja, Spain). Supplied per kilogram of feed: vitamin A (E-672) 10,000 UI; vitamin D_3 (E-671) 2 000 UI; vitamin E (alfa-tocopherol) 25 mg; vitamin B_1 1.5 mg; vitamin B_2 3.5 mg; vitamin B_6 2.4 mg; vitamin B_{12} 20 μg; vitamin K_3 1.5 mg; calcium panthotenate 14 mg; nicotinic acid 20 mg; folic acid 0.5 mg; biotin 50 μg; Fe (E-1) (from $FeSO_4{\cdot}H_2O$) 120 mg; I (E-2) (from $Ca(IO3)_2$) 0.75 mg; Co (E-3) (from $2CoCO_3{\cdot}3Co(OH)_2{\cdot}H_2O$) 0.6 mg; Cu (E-4) (from $CuSO_4{\cdot}5H_2O$) 6 mg; Mn (E-5) (from MnO) 60 mg; Zn (E-6) (from ZnO) 110 mg; Se (E-8) (from Na_2SeO_3) 0.37 mg.
[6] Product of Itpsa (Barcelona, Spain). It contains 56% of antioxidant substances (butylated hydroxytoluene + propyl gallate) and synergistic (Citric acid 14% + authorised support).
[7] Nutrient composition values correspond to the analysed values except for ME and lysine which were estimated according to INRA tables (Sauvant et al., 2004).

(Honeywell, Charlotte, NC, USA) according to Folch et al. (1957) and transmethylated with boron trifluoride (Sigma Aldrich, St. Louis, MO, USA) and potassium hydroxide 0.5 M (PanReac, Barcelona, Spain) in methanol according to Morrison and Smith (1964). Fatty acids were determined by gas chromatography (Agilent 6890N, Boston, MA, USA) using a capillary column (0.25 mm × 0.25 μm × 30 m; DB23, Agilent, Bellefonte, PA, USA) and a flame ionisation detector. A temperature gradient with an initial temperature of 170 °C increased at a rate of 2.5 °C/min until 210 °C followed by another increase at a rate of 5 °C/min to 240 °C, where it remained for 5 min. Injector and detector temperatures were set at 250 °C. Injection was in split mode at a ratio of 100.6:1. The carrier gas was helium with a flux of 55.8 mL/min at the column head (Tous et al., 2014). Internal standards were not used due to the presence of C17:0 and C19:0 in the analysed samples. The external standards used were fatty acid methyl esters (FAMEs) Mix C4-C24 (Supelco, Bellefonte, PA, USA), Nonadecanoic acid methyl ester (Sigma Aldrich, St. Louis, MO, USA), cis-7,10,13,16-Docosatetranoic acid methyl ester (Sigma Aldrich, St. Louis, MO, USA), cis-4,7,10,13,16,19-Docosahexaenoic acid (Sigma Aldrich, St. Louis, MO, USA) and methyl all-cis-5,8,11,14,17-eicosapentaeonate (Sigma Aldrich, St. Louis, MO, USA). Colostrum and milk FA contents were quantified from C12:0 and expressed as mg of FA per g of extracted fat.

Metabolomic analysis of oxylipins

Three colostrum and three milk samples from each treatment were chosen at random, and sixty-five oxylipins were quantified. Aliquots of 1 mL of colostrum or milk were mixed with 0.1 mL of

Table 2
Fatty acid composition of the gestation and lactation sow diets and piglets creep feed.[1]

	Gestation		Lactation		Creep feed	
	Control	n-3 LCFA	Control	n-3 LCFA	Control	n-3 LCFA
Fat (g/kg feed)	35.3	34.6	54.4	55.0	45.3	52.1
Fatty Acids (mg FA/g fat)						
C14:0	4.23	16.8	5.12	12.8	8.34	26.1
C15:0	0.55	1.61	0.38	1.05	0.63	2.10
C15:1	ND	ND	ND	ND	ND	ND
C16:0	146	130	136	127	143	130
C16:1	11.4	18.7	11.2	17.4	11.7	26.5
C17:0	1.15	2.50	1.52	2.29	1.60	3.17
C18:0	37.9	22.3	47.7	39.1	47.8	30.1
C18:1 n-7	10.6	13.4	13.5	14.8	13.4	17.1
C18:1 n-9 *cis*	200	130	242	199	228	139
C18:1 n-9 *trans*	0.70	0.36	1.00	0.64	1.29	0.66
C18:1 n-11 *cis*	0.13	0.37	0.40	0.88	0.71	1.41
C18:2 n-6 *cis*	293	256	237	214	214	167
C18:3 n-3 *cis*	20.1	21.3	14.5	15.3	15.1	17.2
C18:4 n-3	ND	0.56	ND	0.42	ND	0.86
C20:1 n-9 *cis*	4.18	8.00	3.90	5.85	13.4	17.1
C20:2 n-6 *cis*	1.74	0.98	2.33	1.63	2.58	1.04
C20:3 n-3 *cis*	0.23	0.33	0.40	0.55	0.49	0.56
C20:4 n-6	0.92	2.64	1.54	2.47	1.76	3.85
C20:5 n-3	ND	26.7	ND	18.8	ND	41.3
C22:4 n-6	ND	1.60	ND	1.23	ND	1.89
C22:5 n-3	0.49	3.49	0.63	2.24	0.56	5.77
C22:6 n-3	0.49	23.2	0.49	16.5	0.53	36.0
C23:0	0.48	0.48	0.54	0.38	0.51	1.80
C24:0	1.56	1.50	1.40	1.33	1.11	1.20
C24:1 n-9 *cis*	0.76	1.44	0.50	0.98	4.31	8.50
Minor FA[2]	5.28	8.46	5.36	6.67	6.61	10.6
SFA	197	182	197	192	209	203
MUFA	227	173	272	243	260	196
PUFA	317	338	258	277	236	276
n-3	21.3	75.7	16.0	54.0	16.7	102
n-6	296	263	242	223	219	175
n-6:n-3	13.9	3.47	15.1	4.13	13.2	1.72

FA, fatty acid; LCFA, long-chain fatty acid; MUFA, monounsaturated fatty acid; ND, non-detected; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
[1] FA quantification results are reported from C12:0.
[2] Minor FAs include: C12:0, C14:1 n-9 *cis*, C18:2 n-6 *trans*, C18:3 n-6 *cis*, C19:0, C20:0, C20:3 n-6, C21:0, C22:0, C22:1, C22:2 n-6 *cis*, and C22:3 n-3.

internal standard mixture prepared in methanol (butylated hydroxytoluene 0.001 M). A volume of 0.9 mL of methanol (butylated hydroxytoluene 0.001 M) was added to the samples and incubated 30 min at −20 °C. Samples were centrifuged, and the supernatant was recovered and diluted with 10 mL of a 0.1% of formic acid (Merck, Darmstadt, Germany) in Milli-Q water (Millipore, Burlington, MA, USA). A clean-up was applied using Oasis PRIME HLB cartridges (1 cc Vac Cartridge, 30 mg sorbent; Waters Corporation, Milford, MA, USA) eluting twice with 0.6 mL of acetonitrile (Merck, Darmstadt, Germany): methanol (9:1, v/v). The elute was evaporated to dryness under a stream of nitrogen and reconstituted in 0.1 mL of Milli-Q water:methanol (1:1, v/v) (Ostermann, 2017).

An Ultra HPLC 1290 Series coupled to a triple quadrupole mass spectrometer 6490 series instrument (Agilent, Santa Clara, CA, USA) with an analytical column Eclipse XDB C18 1.8 μL (2.1 × 100 mm) (Agilent, Santa Clara, CA, USA) was used to analyse the extracts. The chromatographic separation was performed with a gradient elution using Milli-Q water (0.01% acetic acid (Merck, Darmstadt, Germany)) and acetonitrile:methanol (85:15, v/v) as a mobile phase at a flow rate of 0.4 mL/min and 45 °C. The injection volume was 10 μL (4 °C). The electrospray source ionisation was in negative mode, and the acquisition was performed in dynamic Multiple Reaction Monitoring.

Identification of oxylipins with the corresponding standard was limited because these standards are not commercially available. Therefore, for tentative identification, published data about chromatographic behaviour on C18 columns together with mass spectrometry confirmed oxylipins providing the molecular ion $[M–H]^-$ and fragmentation patterns using electrospray source ionisation in negative mode were used (Astarita et al., 2015; Zhang et al., 2015; Ostermann, 2017). Also published data in relation to the main oxylipin in the studied matrices were used for identification purposes (Bruins et al., 2013; Mavangira et al., 2015). The linear calibration curves used to quantify oxylipins were constructed from available commercial standards using internal standard correction. Internal standard was selected based on chromatographic behaviour criteria. For the compounds with non-available commercial standard, a calibration curve of similar compound was used to perform a tentative identification (Serhan et al., 2006; Isobe, et al., 2012). The available oxylipin analytical standards used were SPM D-series LC-MS Mixture, Lipoxin LC-MS Mixture, EPA Oxylipin LC-MS Mixture, Primary COX and LOX LC-MS Mixture, Leukotriene B4 Pathway LC-MS Mixture, Linoleic Acid Oxylipins LC-MS Mixture, SPM E-series LC-MS Mixture, ALA and GLA Oxylipin LC-MS Mixture, 10(s),17(s)-DiHDHA, 20-HETE, (±)11(12)-DiHET and 8-iso Prostaglandin F2α (Cayman chemicals, Ann Arbor, MI, USA). The stable isotope labelled standards were Resolvin D1-d5, Lipoxin A4-d5, Deuterated Linoleic Acid Oxylipins LC-MS Mixture, Deuterated Primary COX and LOX LC-MS Mixture, Leukotriene B4-d4 (Cayman chemicals, Ann Arbor, MI, USA).

Immune indicators

Different sandwich ELISA kits were employed for the quantitative measurement of Ig and cytokines in all colostrum and milk samples according to the manufacturer's instructions. IgG, IgA and IgM were analysed through Pig IgG ELISA Kit (E101-104; Bethyl Laboratories, Montgomery, Tx, USA), Pig IgA ELISA Kit (ab190536; Abcam, Cambridge, UK) and Pig IgM ELISA Kit (ab190537; Abcam, Cambridge, UK). The cytokine interleukin 1β (**IL1β**), interleukin 6 (**IL6**), interleukin 10 (**IL10**) and tumour necrosis factor α (**TNFα**) were quantified through Pig IL-1β ELISA Kit (ab100754; Abcam, Cambridge, UK), Pig IL-6 ELISA Kit (ab100755; Abcam, Cambridge, UK), Swine IL-10 ELISA Kit (KSC0101/KSC0102; Invitrogen, Carlsbad, CA, USA) and Pig TNF-α ELISA Kit (ab100756; Abcam, Cambridge, UK), respectively. In all kits, the colorimetric reaction was performed with a specific biotinylated secondary antibody and the addition of a streptavidin-conjugated horseradish peroxidase that catalyses the chromogenic substrate 3,3′,5,5′-tetramethylbenzidine. The absorbance was measured at 450 nm. The sample concentrations were determined by comparing the optical density of the samples to a standard curve. Precision values of all kits are given in Supplementary Table S1.

Statistical analysis

The MIXED procedure of SAS software (SAS/STAT 14.1; SAS Institute Inc., Cary, NC, USA) was used to perform the analysis of variance of the different continuous variables and the GLIMMIX procedure of SAS software was used for discrete variables (number of piglets born, number of piglets alive/death or mummies). The model included dietary treatment as fixed effect and batch as random effect. Sow BW at the beginning of the experiment and parity were initially introduced in the model as covariates. However, parity had no significant effect and it was removed from the statistical analysis. For all data at weaning, the variable days of lactation was included in the model as covariate. A square root transformation $(\sqrt{X+0.5})$ of stillborn piglets, deaths and mummies was performed to normalise the data, but least squares means of the original data are presented in tables. Similarly, a logarithmic transformation ($\log_{10}(X + 1)$) of the oxylipin concentration values was performed and means of original data are presented in supplementary tables. When the limit of detection was not reached, the missing values were replaced by 1/5 of the minimum positive value of each variable. Data suspected to be outliers were tested using Kolmogrov-Smirnov test, and values were excluded if $P < 0.01$. Results were expressed as least squares means ± RMSE, except oxylipins that were expressed as means ± RMSE. Differences were considered significant at $P < 0.05$, while those at $P < 0.1$ are reported as tendencies.

MetaboAnalyst 5.0 (http://www.metaboanalyst.ca, Alberta, CA, USA) was used to perform principal component analysis of FA and oxylipins and the heatmap of oxylipins. Graphical representations in bar graph format also were performed through GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Sow's weight and feed intake

The growth and feed intake of sows are presented in Table 3. Sow's BW at insemination (P = 0.159), 107 days of gestation (P = 0.314) and at weaning (P = 0.830), and the average daily gain during gestation (P = 0.315) and lactation (P = 0.559) did not differ between treatments. While sows fed with control diet gained 66.8 ± 2.8 kg during gestation and lost 19.5 ± 3.1 kg during lactation, sows fed with n-3 LCFA diet gained 63.2 ± 2.7 kg during gestation and lost 20.4 ± 3.2 kg during lactation. Finally, average daily feed intake also did not differ between treatments during gestation (P = 0.694) or lactation (P = 0.621).

Litter characteristics and piglet's weight and feed intake during lactation

The number of piglets born, piglets born alive/death, mummies, and piglet's weight and feed intake are presented in Table 4. Litter characteristics at birth did not show any significant difference between treatments, and only a tendency to reduce mummies in n-3 LCFA sows was observed (tendency at P = 0.093). At 24 h after birth, the average number of piglets still alive per litter decreased in the n-3 LCFA group (P = 0.003) and this difference was maintained at day 20 of lactation and at weaning (tendency at P = 0.052 and P = 0.093, respectively). In terms of the average litter

Table 3
Effect of dietary fish oil on the growth and feed intake of gestating and lactating sows.[1]

	Control (n = 18)	n-3 LCFA (n = 18)	RMSE	P value
Days of gestation	116	116	1.05	0.612
Days of lactation	26.8	27.0	1.02	0.681
Average BW (kg)				
Insemination	213	225	24.7	0.159
End of gestation	286	282	10.2	0.314
Day after farrowing	264	265	10.2	0.703
At weaning	245	246	13.5	0.830
Average daily gain (kg)				
Gestation	0.57	0.54	0.09	0.315
Lactation	−0.79	−0.89	0.47	0.559
Total	0.19	0.19	0.10	0.835
Average daily feed intake (kg)				
Gestation	2.78	2.78	0.05	0.694
Lactation	5.59	5.45	0.81	0.621

LCFA, long-chain fatty acid.
[1] Values are least squares means ± RMSE.

Table 4
Effect of dietary fish oil on the litter characteristics and growth performance of suckling piglets.[1]

	Control (n = 18)	n-3 LCFA (n = 18)	RMSE	P value
At birth[2]				
Average total born	15.2	14.3	3.92	0.533
Born alive	14.8	13.8	3.67	0.442
Stillborn	0.30	0.46	0.28	0.468
Mummies	0.41	0.10	0.24	0.093
Average litter weight (kg)	19.6	18.9	3.63	0.596
Average piglet BW (kg)	1.37	1.37	0.24	0.953
SD piglet BW (kg)	0.25	0.26	0.06	0.716
24 h after birth[3]				
Average still alive	13.5	11.8	1.54	0.003
Average of deaths 24 h	0.39	0.90	0.41	0.189
Average litter weight (kg)	19.5	18.2	2.78	0.207
Average piglet BW (kg)	1.42	1.54	0.23	0.161
SD piglet BW (kg)	0.31	0.32	0.09	0.574
20 days after birth				
Average still alive	11.7	10.9	1.13	0.052
Average litter weight (kg)	71.4	72.7	8.41	0.669
Litter ADG (24 h → 20 d) (kg)	2.57	2.68	0.35	0.392
Average piglet BW (kg)	6.06	6.68	0.68	0.013
SD piglet BW (kg)	1.22	1.19	0.38	0.797
Piglet ADG (24 h → 20 d) (kg)	0.24	0.27	0.03	0.010
At weaning				
Average still alive	11.6	10.9	1.17	0.093
Average of deaths lactation	2.37	1.80	0.58	0.420
Average litter weight (kg)	92.5	93.5	10.2	0.794
Litter ADG (24 h → W) (kg)	2.77	2.85	0.33	0.472
Average piglet BW (kg)	7.91	8.50	0.87	0.058
SD piglet BW (kg)	1.48	1.46	0.47	0.913
Piglet ADG (24 h → W) (kg)	0.25	0.27	0.03	0.072
Piglet creep feed intake (kg)	0.29	0.31	0.07	0.471

ADG, average daily gain; LCFA, long-chain fatty acid; W, weaning.
[1] Values are least squares means ± RMSE.
[2] One sow from n-3 LCFA diet farrowed out of the scheduled time, without supervision, and litter characteristics at birth were not possible. Two sows from n-3 LCFA diet gave birth to less than six piglets and were excluded for the data analysis of litter characteristics at birth.
[3] Adoptions were completed within 24 h after birth, and the 24 h recordings were considered as the initial values for the litter characteristics and growth performance of suckling piglets.

weight, no difference was observed between treatments. However, the average of individual piglet's BW at day 20 after birth (P = 0.013) and at weaning (tendency at P = 0.058), and piglet's average daily gain between 24 h and day 20 after birth (P = 0.010) and between 24 h and weaning (tendency at P = 0.072) were increased in piglets from n-3 LCFA sows compared to piglets from control sows. Piglet creep feed disappearance did not differ between treatments (P = 0.471).

Fatty acid profile

The changes caused by sow diet's fat source on colostrum and milk FA profile are shown in Table 5. In colostrum, the fish oil did not change the fat content compared to control diet. In terms of FAs, no changes by dietary treatment were observed in total saturated FAs or monounsaturated FAs. However, some saturated FAs such as C14:0 (P < 0.001), C15:0 (P < 0.001), C17:0 (P = 0.049), C23:0 (P < 0.001), and C24:0 (P = 0.040), and some monounsaturated FAs such as C15:1 (P < 0.001), C16:1 (P = 0.014) and C18:1 n-11 *cis* (P = 0.034) were increased and only a reduction of the monounsaturated FA C18:1 n-9 *trans* (P < 0.001) was observed in n-3 LCFA-treated sows. In reference to polyunsaturated FAs, an increase in total n-3 FAs (P < 0.001), α-linolenic acid (C18:3 n-3 *cis*) (P = 0.003), stearidonic acid (C18:4 n-3) (P < 0.001), eicosatrienoic acid (C20:3 n-3 *cis*) (P < 0.001), EPA (P < 0.001), docosapentaenoic acid (C22:5 n-3) (P < 0.001), and DHA (P < 0.001) was observed in n-3 LCFA-treated sows compared to control. In contrast, the n-6 family was barely affected by dietary treatment since only a reduction of arachidonic acid (C20:4 n-6) was observed in fish oil-treated sows (P = 0.003). Consequently, total polyunsaturated FAs (P = 0.020) were increased and the n-6:n-3 ratio was significantly reduced (P < 0.001) in colostrum from n-3 LCFA fed sows.

In milk, fat content, total saturated FAs and monounsaturated FAs were not affected by dietary treatments, and only trends to increase C15:0 (P = 0.065) and C23:0 (P = 0.061), an increase of C15:1 (P < 0.001) and C24:1 n-9 (P = 0.050) and a reduction of C18:1 n-9 *trans* (P = 0.005) were observed in samples from the n-3 LCFA sows. In addition, sows fed the n-3 LCFA diet had a higher concentration of n-3 polyunsaturated FAs (P < 0.001) mainly due to increases in stearidonic acid (P < 0.001), EPA (P < 0.001), docosapentaenoic acid (P = 0.002), and DHA (P < 0.001). The n-6 FAs were not changed and only arachidonic acid concentration was reduced (P = 0.019) and the adrenic acid (C22:4 n-6) (P = 0.027) concentration increased by dietary fish oil, which resulted in a reduction of the n-6:n-3 ratio (P < 0.001) without an increase of total polyunsaturated FAs.

Principal component analysis allowed to observe a different distribution of the samples according to sample type (colostrum or milk) and diet (control or n-3 LCFA) (Fig. 1, A). EPA was the FA with the highest contribution in explaining the principal component analysis distribution.

Oxylipin profile

In colostrum, most of the differences observed between treatments were for oxylipins derived from EPA or DHA, and their con-

Table 5
Colostrum and milk fatty acid profile from sows fed control or n-3 LCFA diet.[1,2]

	Colostrum				Milk			
	Control (n = 18)	n-3 LCFA (n = 17)	RMSE	P value	Control (n = 18)	n-3 LCFA (n = 18)	RMSE	P value
Fat (g/kg sample)	64.2	56.6	18.8	0.244	73.8	71.1	19.0	0.672
Fatty acid (mg FA/g fat)								
C14:0	8.42	13.1	3.44	<0.001	18.1	19.3	5.63	0.545
C15:0	0.71	1.14	0.33	<0.001	0.44	0.57	0.21	0.065
C15:1	0.35	0.57	0.13	<0.001	0.17	0.33	0.11	<0.001
C16:0	135	153	40.3	0.212	152	152	45.5	0.970
C16:1	15.9	20.4	4.96	0.014	47.1	49.0	17.6	0.758
C17:0	1.75	2.21	0.64	0.049	0.87	0.94	0.33	0.506
C18:0	35.4	39.2	12.1	0.369	21.0	19.8	6.70	0.588
C18:1 n-7	15.7	17.9	4.89	0.196	12.2	12.5	4.45	0.843
C18:1 n-9 *cis*	181	188	70.0	0.741	186	178	63.0	0.723
C18:1 n-9 *trans*	1.26	0.76	0.39	<0.001	1.16	0.75	0.40	0.005
C18:1 n-11 *cis*	0.87	1.24	0.48	0.034	0.84	0.97	0.27	0.175
C18:2 n-6 *cis*	132	148	41.2	0.282	76.2	74.5	24.4	0.841
C18:3 n-3 *cis*	6.96	9.79	2.57	0.003	3.91	4.32	1.53	0.427
C18:4 n-3	0.05	0.90	0.55	<0.001	0.03	0.38	0.10	<0.001
C20:1 n-9 *cis*	1.38	1.80	0.96	0.219	2.21	2.17	1.06	0.925
C20:2 n-6 *cis*	3.04	2.61	1.30	0.346	2.19	1.84	0.80	0.211
C20:3 n-3 *cis*	0.87	1.26	0.27	<0.001	0.58	0.65	0.22	0.375
C20:4 n-6	7.47	4.98	2.22	0.003	2.70	1.85	1.02	0.019
C20:5 n-3	0.08	8.22	1.60	<0.001	0.14	3.22	0.83	<0.001
C22:4 n-6	0.76	0.83	0.15	0.182	0.17	0.36	0.23	0.027
C22:5 n-3	2.34	9.93	2.23	<0.001	0.60	1.55	0.84	0.002
C22:6 n-3	0.70	11.7	2.74	<0.001	0.37	3.83	0.95	<0.001
C23:0	0.09	0.74	0.18	<0.001	0.06	0.13	0.11	0.061
C24:0	0.95	1.06	0.15	0.040	0.92	1.32	0.93	0.206
C24:1 n-9 *cis*	0.76	0.75	0.17	0.876	0.72	0.56	0.23	0.050
Minor FA[3]	6.59	6.56	1.67	0.958	6.35	6.30	1.74	0.932
SFA	184	212	56.0	0.163	197	196	57.4	0.991
MUFA	218	232	69.2	0.556	252	246	80.1	0.839
PUFA	159	202	51.5	0.020	88.6	94.3	29.1	0.569
n-3	11.0	41.8	8.52	<0.001	6.36	14.7	3.46	<0.001
n-6	148	161	44.3	0.406	83.2	83.8	26.9	0.940
n-6:n-3	13.3	3.88	0.58	<0.001	13.0	5.70	0.79	<0.001

FA, fatty acid; LCFA, long-chain fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
[1] Values are least squares means ± RMSE.
[2] FA quantification results are reported from C12:0.
[3] Minor FAs include: C12:0, C13:0, C14:1 n-9 *cis*, C18:2 n-6 *trans*, C18:3 n-6 *cis*, C19:0, C20:0, C20:3 n-6, C21:0, C22:0, C22:1, C22:2 n-6 *cis*, and C22:3 n-3. C21:0 and C22:3 n-3 have not been detected in colostrum.

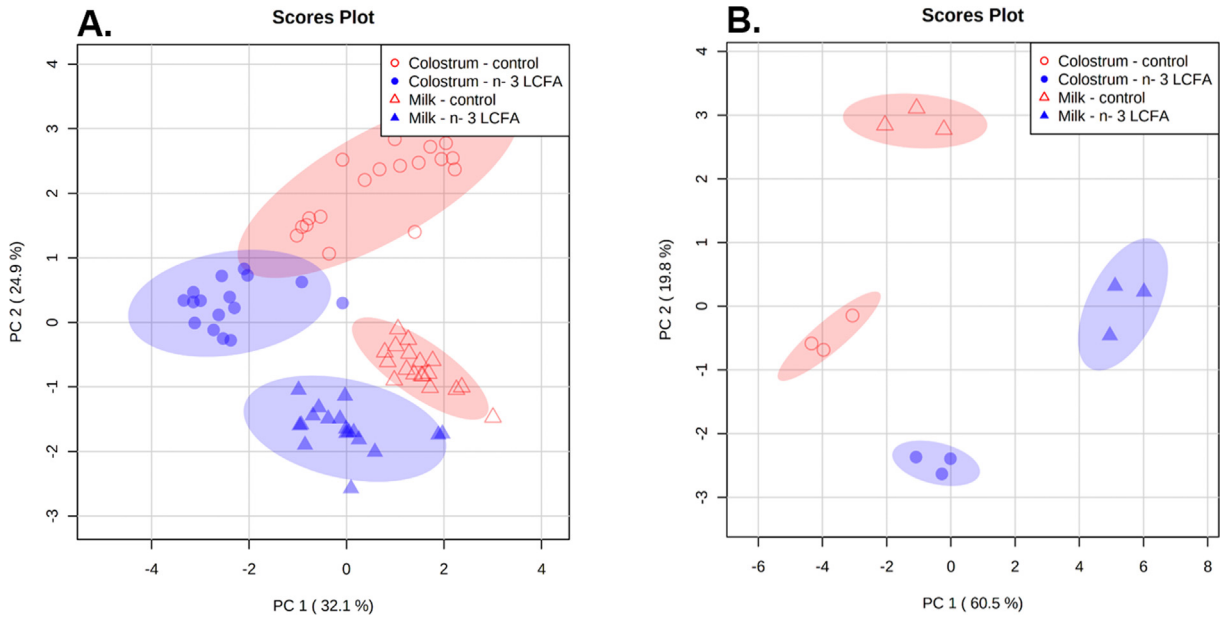

Fig. 1. Fish oil source rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in sow diets modifies fatty acid (FA) and oxylipins in colostrum and milk. Principal component analysis 2 dimensions score plot of FA profile (A) and oxylipins (B) of colostrum and milk samples. In terms of FA profile (A) values are means of 18 samples per treatment, except for colostrum n-3 Long-Chain Fatty Acids (n-3 LCFA) diet (n = 17). In terms of oxylipins profile (B) values are means of 3 samples per treatment.

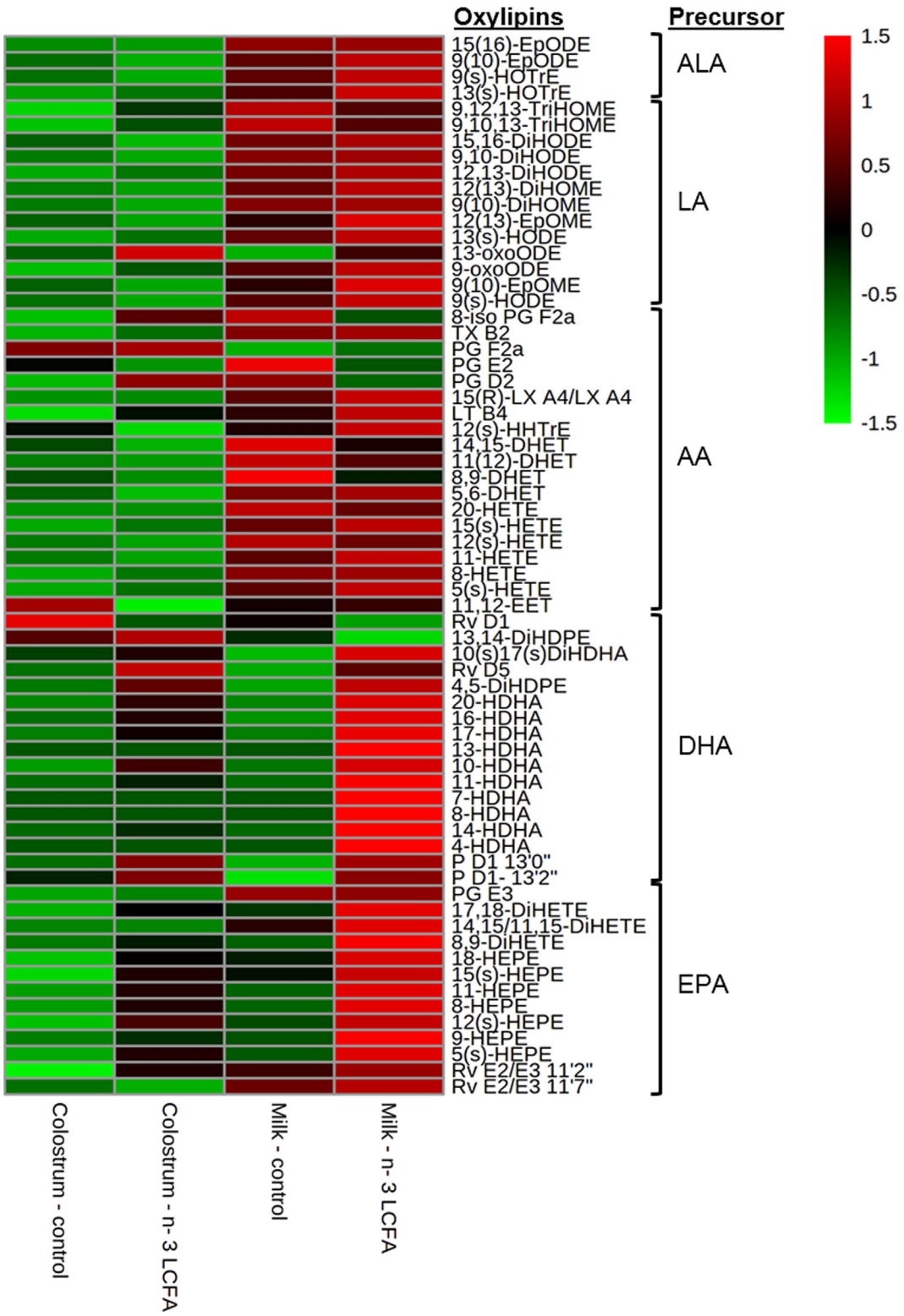

Fig. 2. Eicosapentaenoic acid (EPA)- and docosahexaenoic acid (DHA)-derived oxylipins are increased in colostrum and milk from fish oil-fed sows. Values are means of 3 samples per treatment. AA, arachidonic acid; ALA, α-linolenic acid; DiHDPE, dihydroxy-docosapentaenoic acid; DiHDHA, dihydroxy-docosahexaenoic acid; DiHETE, dihydroxy-eicosatetraenoic acid; DiHODE, dihydroxy-octadecadienoic acid; DiHOME, dihydroxy-octadecenoic acid; DHET, dihydroxy-eicosatrienoic acid; EET, epoxy-eicosatrienoic acid; EpODE, epoxy-octadecadienoic acid; EpOME, epoxy-octadecenoic acid; HDHA, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HHTrE, hydroxy-heptadecatrienoic acid; HODE, hydroxy-octadecadienoic acid; HOTrE, hydroxy-octadecatrienoic acid; LA, linoleic acid; LCFA, long-chain fatty acid; LT, Leukotriene; Lx, Lipoxin; oxoODE, oxo-octadecadienoic acid; P, Protectin; PG, Prostaglandin; Rv, Resolvin; TX, Thromboxane; TriHOME, trihydroxy-octadecenoic acid.

Table 6
Colostrum and milk immune indicator from sows fed control or n-3 LCFA diet.[1]

	Colostrum				Milk			
	Control (n = 18)	n-3 LCFA (n = 17)	RMSE	P value	Control (n = 18)	n-3 LCFA (n = 18)	RMSE	P value
Immunoglobulins (mg/mL)								
IgG	186	149	141	0.461	0.65	0.74	0.39	0.504
IgA	4.48	2.01	7.12	0.316	3.20	4.42	2.21	0.111
IgM	1.42	1.03	1.22	0.354	1.54	1.55	0.66	0.966
Cytokines (ng/mL)								
IL1β	25.1	31.1	13.0	0.197	6.12	7.09	8.25	0.729
IL6	137	187	190	0.448	7.00	6.19	1.90	0.243
IL10	3.70	11.4	20.0	0.298	0.10	0.09	0.01	0.059
TNFα	1.15	1.92	2.25	0.334	0.16	0.15	<0.01	0.011

IgA, immunoglobulin A; IgG, Immunoglobulin G; IgM, immunoglobulin M; IL-1 β, interleukin 1β; IL6, interleukin 6; IL10, interleukin 10; TNF-α, tumour necrosis factor α.
[1] Values are least squares means ± RMSE.

centrations were increased when sows were fed the fish oil diet (Supplementary Table S2). The EPA-derived oxylipins that increased by the n-3 LCFA diet were 17,18-dihydroxy-EPA (P = 0.004); 5(s)-, 8-, 11-, 12(s)-, 15(s)- and 18-hydroxy-EPA (P < 0.001, P = 0.002, P = 0.013, P = 0.030, P = 0.005 and P = 0.006, respectively) and Resolvin E2/E3 (P = 0.002). In addition, the DHA-derived oxylipins increased by n-3 LCFA are mainly hydroxyl-DHA metabolites such as 10-, 16- and 20-hydroxy-DHA (P < 0.001, P = 0.036 and P = 0.012, respectively); 4,5-dihydroxy-docosapentaenoic acid (P = 0.013); Resolvin D5 (P = 0.009) and Protectin D1 (tendency at P = 0.076). Prostaglandin D2 (tendency at P = 0.058) derived from arachidonic acid was also increased when fish oil was included in the sow diet.

For the sows fed the n-3 LCFA diet, 24 oxylipin concentrations were increased (P < 0.05) and 6 tended to increase (P < 0.1) in milk (Supplementary Table S3). Among these, the EPA-derived oxylipins that increased were as follows: 8, 9-, 14,15-/11,15- and 17,18-dihy droxy-eicosatetraenoic acid (P = 0.002, P = 0.003 and P = 0.006, respectively); 5(s)-, 8-, 9-, 11-, 12(s)-, 15(s)- and 18-hydroxy-EPA (P = 0.001, P = 0.005, P = 0.002, P = 0.003, P = 0.005, P = 0.007 and P = 0.003, respectively) and Resolvin E2/3 (P = 0.050). The DHA-derived oxylipins that increased were as follows: 10(s),17 (s)-dihydroxy-DHA (also known as Neuroprotectin D1; tendency at P = 0.069); 4,5-dihydroxy-docosapentaenoic (P = 0.002); 4-, 8-, 10-, 11-, 13-, 14-, 16-, 17- and 20-hydroxy-DHA (P < 0.001, P = 0.002, P < 0.001, P = 0.012, P < 0.001, P = 0.001, P = 0.002, P = 0.002 and P < 0.001, respectively) and Protectin D1/Protectin D1- (P = 0.016 and P = 0.024). In addition to the changes in EPA- and DHA-derived oxylipins, 9(10)-epoxy-octadecadienoic acid (tendency at P = 0.064) and 9(s)-hydroxy-octadecatrienoic acid (tendency at P = 0.058), which are derived from α-linolenic acid, and 9(10)- and 12(13)-epoxy-octadecenoic acid (tendency at P = 0.056 and P = 0.038, respectively) and 9(s)-hydroxy-octadecadienoic acid (tendency at P = 0.054), which are derived from linoleic acid (C18:2 n-6) were also increased.

Even though the oxylipin profile of the n-3 LCFA sows is clearly differentiated in the 2D plots obtained through principal component analysis for both, colostrum and milk (Fig. 1, B), these differences could not be explained by changes in any particular oxylipin, rather they were consequence of the overall changes observed in the whole oxylipin profile. Taken together, the colostrum and milk n-3 FA-derived oxylipins were strongly increased by dietary fish oil, and a higher number of oxylipins were modified in milk than in colostrum (Fig. 2). Finally, a schematic overview of the oxylipins increased by the n-3 LCFA diet and their FA precursor is summarised in Supplementary Fig. S1.

Immunological analysis

Colostrum and milk Ig were not significantly affected by dietary treatment (Table 6). IgG concentrations were higher in colostrum than in milk and ranged between 185–212 mg/mL and 0.649–0.737 mg/mL, respectively. However, IgA and IgM concentrations were similar in both, colostrum and milk. IgA and IgM concentrations ranged between 2.01–4.48 mg/mL and 1.03–1.42 mg/mL in colostrum, and between 3.20–4.42 mg/mL and 1.54–1.55 mg/mL in milk, respectively.

Cytokine concentrations in colostrum were not affected by dietary treatment despite being larger than those of milk samples (Table 6). In milk, no changes were observed for IL1β and IL6. However, a trend for reduced IL10 (tendency at P = 0.059) and a reduced TNFα (P = 0.011) was observed in milk from sows fed the n-3 LCFA diet.

Discussion

Colostrum and milk are the first energy and nutrient sources for newborn piglets, which have low energy reserves. In addition, they also play a key role in the transfer of passive immunity (Darragh and Moughan, 1998). For these reasons, colostrum and milk are essential for the preweaning growth of piglets. The reduction of litter size observed in this study is in line with the results reported by Rooke et al. (2001) feeding sows with salmon oil from day 60 of gestation. However, and as summarised in the revision of Tanghe and De Smet (2013), previous studies reported no effect of dietary n-3 polyunsaturated FAs on embryo number, development, size or survival, and the reason for the reduced litter size is still unclear. On the other hand, Rooke et al. (2001) and Laws et al. (2007) found that fish oil supplementation lowered piglet birth weight contrary to the increase of birth weight of piglets from treated litter observed in the current study. This could be related to the fact that smaller litters in n-3 LCFA sows allow their piglets to have more access to colostrum and milk though litter weight was not different throughout lactation.

Piglets are able to digest more than 90% of the lipids present in colostrum and milk (Azain, 2001), thus becoming efficient vehicles to transfer FA (Lauridsen, 2020). Under commercial conditions, sow diets are composed of ingredients that contain considerable amounts of n-6 FAs, the fat source is rich in saturated FAs, and fish oil is not commonly used. Several studies have already observed an increased n-3 FAs concentration in colostrum and milk when an n-3 rich source is used in the maternal diet (Rooke et al., 2001;

Eastwood et al., 2014). In the current study, apart from EPA and DHA, increases for α-linolenic acid, stearidonic acid, eicosatrienoic acid and docosapentaenoic acid in colostrum and stearidonic acid and docosapentaenoic acid in milk were also observed when the sows were fed the fish oil diet, which is in contrast with previous studies that reported no differences for these FAs (Eastwood et al., 2014). Regarding the n-6 polyunsaturated FA family, we observed a decline of arachidonic acid concentration in both, milk and colostrum, when fish oil was added to the sow diet. These results confirm those of Eastwood et al. (2014) in colostrum. This decline in arachidonic acid concentration could be explained by the fact that an n-3 FA diet enrichment is typically accompanied by a decrease in the content of arachidonic acid in different cell types (Calder, 2010). In our study, total n-3 FA increased but saturated FAs, monounsaturated FAs and total n-6 FAs were not affected by dietary treatment in colostrum or milk. We also observed that the n-3 LCFA diet had more impact in colostrum than in milk since a higher number of FAs were altered and the magnitude of the changes was also larger. Regarding EPA and DHA, we observed concentrations of 0.006 mg and 0.045 mg per gram of colostrum in the control, and 0.450 mg and 0.629 mg in the n-3 LCFA samples, respectively. In milk, the concentrations ranged between 0.003 mg and 0.030 mg per gram in the control, and 0.225 mg and 0.265 mg per gram in the n-3 LCFA samples, respectively.

It is well established that polyunsaturated FAs can influence inflammatory processes through a variety of mechanisms, one of them being their oxidation by enzymatic or non-enzymatic pathways and the consequent formation of oxylipins (Calder, 2010). To our knowledge, the presence of these compounds has not been studied in colostrum or sow's milk. Fish oil in sow diets has an impact on FA profile and consequently on oxylipin profile. In addition, the detection of these lipid mediators in colostrum and milk implies a transfer of oxylipins from sow to piglet. In fact, it has been described that these oxylipins influence the coordination of a balanced inflammatory response and that each oxylipin possesses proinflammatory and/or anti-inflammatory functions (Gabbs et al., 2015), which could affect the immune status of piglets. Furthermore, it is known that n-6 polyunsaturated FA-derivate oxylipins tend to have proinflammatory activity whereas n-3 polyunsaturated FA-derivate oxylipins present a low proinflammatory potential or/and anti-inflammatory potential (Calder, 2010). The enhanced concentration of EPA and DHA in colostrum and milk of fish oil-fed sows resulted in increases of hydroxy-EPA or hydroxy-DHA that are directly derived from these LCFAs through the enzymatic pathway involving lipoxygenase (Astarita et al., 2015). Most of these intermediate hydroxy-FAs currently do not have a defined function except those described in specific murine or human cell lines by Gabbs et al. (2015). EPA and DHA also give rise to resolvins and related compounds as protectins through the lipoxygenase or cyclooxygenase pathways (Serhan et al., 2002; Serhan et al., 2008). In the current study, E-series Resolvin E2/3 and D-series Resolvin D5 were increased in the colostrum and milk samples from the n-3 LCFA diet fed sows. In addition, Protectin D1 and Neuroprotectin D1 in milk, and Protectin D1 in colostrum were also increased. The biological effects of resolvins and protectins have been widely examined in several cell cultures and animal models of inflammation, and they have been shown to possess potent anti-inflammatory and inflammation resolving activity (Calder, 2010). Some studies suggest that this increment of EPA- and DHA-derived oxylipins should be accompanied by a decrease in arachidonic acid-derived oxylipins (Calder, 2010). However, the review of Shearer and Walker (2018) mentions that an inclusion of n-3 polyunsaturated FA can increase the availability of some n-6 polyunsaturated FA-derived oxylipins. In the present study, no significant reduction of arachidonic acid-derived oxylipins or any other n-6 FA-derived oxylipins was observed in colostrum or milk from the fish oil-fed animals. In fact, there was a trend to increase Prostaglandin D2 in colostrum, which is an arachidonic acid-derived eicosanoid. Prostaglandin D2 plays an important role in reproduction, especially in the implantation and maintenance of pregnancy (Saito, et al., 2002), which could explain this increase in colostrum (produced during the late stages of gestation) but not in milk. Tanghe and De Smet (2013) suggest that EPA and DHA may regulate gestation length decreasing the synthesis of 2-series Prostaglandins such as Prostaglandin E2 and Prostaglandin F2a. However, no differences in the concentration of these last two Prostaglandins or the gestation length were observed with the fish oil inclusion in the current study. In milk, 9(10) epoxy-octadecadienoic acid and 9(s)-hydroxy-octadecatrienoic acid derived from α-linolenic acid and 9(10)-, 12(13)-epoxy-octadecenoic, 9(s)-hydroxy-octadecadienoic acid derived from linoleic acid were also increased in the n-3 LCFA diet, although no differences in the precursor FAs were detected. Contrary to FA profile, the major changes in oxylipins were observed in milk rather than in colostrum. This could be explained by the high amounts of EPA- and DHA-derived oxylipins found in milk, suggesting a larger oxygenation process of n-3 LCFA.

In addition to the effect that n-3 polyunsaturated FAs can exert on inflammation via changes in the pattern of oxylipins, they also have an impact on the production of cytokines and Ig (Mitre et al., 2005; Calder, 2010; Yao, et al., 2012). In the current study, IgG concentrations were higher than those reported in the literature (Mitre et al., 2005; Leonard et al., 2010; Yao et al., 2012) but no differences between treatments were observed. However, although some studies reported increases in IgG concentration in colostrum and milk with dietary fish oil (Mitre et al., 2005), others have not observed this effect (Leonard et al., 2010). In addition, Yao et al. (2012) reported an increase in colostrum IgG and milk IgM for sow dietary n-6:n-3 ratios of 9:1 in comparison to a 3:1 ratio. These ratios are similar to those used in the present study; however, in the present case, Ig concentrations in colostrum and milk were not altered by the n-6:n-3 ratio of the maternal diet. Such discrepancies among studies deserve to be further investigated. In terms of cytokine production, the fish oil diet did not cause changes in colostrum. However, there is a trend to reduce IL10 and a reduction of TNFα in the milk of animals fed the n-3 LCFA diet. These effects may be due to the greater effect of n-3 LCFA diet on the oxylipin profile in milk than in colostrum. IL10 plays an important anti-inflammatory role inhibiting the production of proinflammatory cytokines (Walter, 2014), and TNFα plays a proinflammatory role since it is an inducer of inflammatory response (Idriss and Naismith, 2000). In the review of Calder (2010) data from in vitro (cell culture) and in vivo (mice and humans) studies showed a lowering effect of proinflammatory cytokine production such as IL1β, IL6 and TNFα by dietary fish oil. However, other studies do not confirm this effect and they refer to a possible dose effect, to technical factors, to the relative contributions of EPA and DHA and even to effects of polymorphisms of certain genes. Moreover, the studies in the review did not focus on the effects of n-3 LCFA in colostrum or milk.

As already mentioned, resolvins and protectins possess potent anti-inflammatory and inflammation resolving activities (Calder, 2010). Resolvin E2/3 is related with the inhibition of proinflammatory cytokine production and the production of anti-inflammatory cytokines in peritonitis studies with human and murine models (see Gabbs et al., (2015) for references). Previous in vitro reports show that protectins inhibit IL1β and TNFα production in human cell lines (see Serhan et al., (2008) for references). Moreover, it has been described that 18-hydroxy-eicosapentaenoic acid, an hydroxy-EPA, and 13- and 17-hydroxy-docosahexaenoic acid, hydroxy-DHA derivates decrease or inhibit TNF-α secretion in

murine or human cell lines (see Gabbs et al., (2015) for references) (Serhan et al., 2002). The decline of TNFα observed in milk is in line with the reported information. In the current study, although not differentially significant, IL1β levels in milk and colostrum were numerically higher for the n-3 LCFA diet than for the control diet. Future studies evaluating a wider range of cytokines and transcription factors that play a role in inflammation could provide a more complete overview of the effects of n-3 LCFA on the immunological profile of colostrum and milk.

Conclusion

Finally, this study provides a complete picture of precursors, intermediate molecules, and final mediators in colostrum and milk when sows are fed control or n-3 LCFA diets, which allows us to conclude that the inclusion of fish oil rich in EPA and DHA in sow diets during gestation and lactation may reduce litter size without affecting total litter weight during lactation, promotes an increase of EPA, DHA and their oxygenated derivatives with anti-inflammatory and inflammation resolving activity in colostrum and milk, and reduces TNFα and IL10 in milk. The implications of the changes observed in the colostrum and milk of sows fed n-3 LCFA for the immune status of the piglets remain to be analysed.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100403.

Ethics approval

IRTA's Ethical Committee on Animal Experimentation approved the use of animals for this experiment in accordance with the Directive 2010/63/EU of 22 September 2010 and according to the recommendation of the European Commission 2007/526/CE, the Spanish guidelines for the care and use of animals in research (B. O.E. number 34, Real Decreto 53/2013) and the regional regulations on the use and handling of experimental animals (Decree 214/97, Generalitat de Catalunya) (project number: 10294).

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available upon request.

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Declaration of interest

The authors report no conflict of interests.

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