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Eicosapentaenoic acid- and docosahexaenoic acid-rich fish oil in sow and piglet diets modifies blood oxylipins and immune indicators in both, sows and suckling piglets

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ABSTRACT

Over the last decades, genetic selection has increased sows' litter size. Consequently, there is a high proportion of piglets born with low weight which are vulnerable. Their viability may potentially be enhanced through early nutrition. The aim of the current study was to evaluate whether including a fish oil rich in eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) in the diets of the sow and piglets was able to increase concentrations of anti-inflammatory molecules in their blood. Thirty-six sows, in four consecutive batches, were randomly assigned to either a control diet with animal fat (15 g/kg in gestation and 30 g/kg in lactation) or an n-3 long-chain fatty acid (**n-3 LCFA**) diet from insemination until the end of lactation. From day 11 of lactation, piglets were also offered a diet containing 30 g/kg of animal fat or n-3 LCFA. To prepare the n-3 LCFA diet, 15 g/kg or 30 g/kg of animal fat in the control diet were replaced by an equivalent amount of solid fish oil for sows and piglets, respectively. All the sows were sampled for serum and plasma at day 108 of gestation and at weaning. Additionally, only for the first batch of sows, blood samples were also obtained at weaning from the two lightest (>800 g) and the two heaviest birth weight piglets in each litter. Serum fatty acids (**FAs**) were quantified by gas chromatography, plasma oxylipins by ultra-HPLC-MS and plasma immunoglobulins (**Ig**) and cytokines by ELISA. The n-3 LCFA diet increased the concentrations of n-3 FAs in gestating and lactating sows and in piglets ($P < 0.001$, $P < 0.001$ and $P = 0.011$, respectively), particularly EPA ($P < 0.001$, $P < 0.001$ and $P < 0.001$, respectively) and DHA ($P < 0.001$, $P < 0.001$ and $P < 0.001$, respectively), and also their oxygenated derivatives. In addition, fish oil increased plasma IgM in gestating and lactating sows ($P = 0.014$ and $P = 0.008$, respectively), interleukin (**IL**) 6 in sows at weaning ($P = 0.012$), and IL1β in piglets ($P = 0.018$). Birth BW of piglets, regardless of diet, slightly influenced some of the n-6-derived oxylipins. In conclusion, fish oil addition in diets increased the blood concentrations of n-3 FAs and their oxygenated derivatives, some of which have anti-inflammatory activity, in gestating and lactating sows and piglets, IgM in gestating and lactating sows, IL6 in lactating sows and IL1β in piglets.

Implications

This study shows that the inclusion of fish oil in sow diets during gestation and lactation and in creep feed influences blood serum fatty acid and plasma oxylipin profiles in sows at late gestation and weaning and piglets at weaning. The observed increases in n-3 fatty acids and their oxygenated derivatives have been described to play an anti-inflammatory role. Therefore, fish oil in the diets of sows could improve the immune status of piglets, particularly during the postweaning period which, from an immunocompetence point of view, is the most critical period in swine production.

Introduction

In the current swine production system, genetic selection of sows has focused in increasing their litter size, and this has resulted in a reduction of the average birth weight of piglets and an increase in the proportion of piglets born with low weight

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(<1.0 kg birth weight) (Blavi et al., 2021). Low birth weight (**LBW**) piglets are characterised by a particularly poor thermoregulation ability and generally have problems to achieve and adequate colostrum intake (Edwards and Baxter, 2015). For these reasons, LBW piglets are most vulnerable and have a greater incidence of mortality than their heavier littermates (Farmer and Edwards, 2022).

A potential approach to improve LBW piglets' viability is through sow nutrition. Dietary polyunsaturated fatty acids (**FAs**) are commonly present in swine diets and are used as an energy source (Rosero et al., 2016). However, they also play important roles as structural components of the cell membrane, metabolic substrates in biochemical pathways, cell-signalling molecules and immune modulators (Liu, 2015). It is well established that polyunsaturated FAs can influence the immune status through a variety of mechanisms (Lauridsen, 2020; Cader, 2012). One of them is through the formation of oxygenated derivatives (oxylipins) (Balvers et al., 2012) and their subsequent effect on cytokines synthesis (Calder, 2010). Concretely, oxylipins exert multiple functions in the modulation of health and disease in mammals (Gabbs et al., 2015; Christie and Harwood, 2020). They are important mediators of the PUFA effects in the body, and their formation is associated with the nature of dietary FAs.

Under commercial conditions, dietary n-6:n-3 FA ratios in sow and piglet diets are very high, as their main ingredients are commonly rich in n-6 FAs and contain supplemental fat sources that are rich in saturated FAs and have very low levels of n-3 FAs. Dietary n-6 and n-3 polyunsaturated FAs have different effects on the immune system; while the n-6 family are precursors of oxylipins with proinflammatory potential, the n-3 family are precursors of anti-inflammatory and inflammation resolving oxylipins (Calder, 2010). Oxylipins are the major lipid mediators for the polyunsaturated FA effects in the body (Gabbs et al., 2015) and are considered to be critically involved in neonatal physiology (Wu et al., 2016).

Considering the anti-inflammatory effects associated with n-3 FAs, it was hypothesised that n-3 long-chain FAs (**n-3 LCFA**) in sow and piglet diets would increase n-3 polyunsaturated FAs and their oxygenated derivatives, which in turn would influence immune indicators in different biological matrices from sows and piglets in different points of the productive swine cycle. In the scope of this project, we previously reported that the inclusion of fish oil in sow diets promoted an increase of n-3 polyunsaturated FAs and their oxygenated derivatives in colostrum and milk, and reduced tumour necrosis factor α (**TNFα**) and interleukin (**IL**) 10 in milk (Llauradó-Calero et al., 2021). This suggests that dietary fish oil may also influence the FA content, oxylipin profile and immune status of suckling piglets. This is particularly relevant since piglets are born with poor energy reserves and are devoid of immune protection (Le Dividich et al., 2005).

Therefore, we analysed blood samples from sows and piglets in our previous study with the aim of determining how the inclusion of fish oil, rich in eicosapentaenoic acid (**EPA**) (C20:5n-3) and docosahexaenoic acid (**DHA**) (C22:6n-3), in sow and piglet diets influence FA and oxylipin profiles, and systemic immune indicators of sows at the end of gestation and at weaning and particularly in piglets at weaning.

Material and methods

Animals, housing and experimental design

Thirty-six sows from four consecutive batches (same animals and experimental set-up as Llauradó-Calero et al. (2021) were used. The sows were randomly assigned to either a control diet or an n-3 LCFA-rich diet from insemination (day 0) until the end of lactation (±28 days of lactation). In the first batch of sows (12 sows), the two piglets with lightest birth weight (>800 g) and the two piglets with highest birth weight in each litter were selected for blood sampling. Cross fostering (to standardise the litter size to 12 piglets) was exclusively conducted among sows belonging to the same experimental treatment and within the first 24 h postfarrowing. At day 11 of lactation, creep feed (with or without n-3 LCFA) was also offered to piglets in accordance with the maternal diet.

Sows were allocated to individual stalls from insemination to pregnancy confirmation, followed by group housing in a gestation barn until 1 week before farrowing, when 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 temperature inside the building was automatically set at 24 °C at farrowing and reduced by 0.5 °C per week until weaning. Ventilation was via single, variable-speed fans linked to temperature sensors. Sows were fed via individual feed hoppers and piglets from snap-in round feeders. Water was provided *ad libitum* from independent nipple drinkers for sows and piglets.

Experimental diets

Gestation and lactation diets were formulated (iso-nutritive between dietary treatments) according to FEDNA specifications (de Blas et al., 2013). In the control diets, animal fat was included at 15 g/kg (gestation phase) and 30 g/kg (lactation phase). In the n-3 LCFA diets, animal fat was totally (gestation phase) or half replaced (lactation phase) by solid fish oil (Lipomega®; V&S Asociados, Madrid, Spain). Creep feed contained 30 g/kg of animal fat in the control diet, and this was totally replaced by solid fish oil in the n-3 LCFA diet. The ingredient and nutrient composition of the diets has already been described in Llauradó-Calero et al. (2021).

Sows were feed-restricted during gestation at 3 kg/day and after farrowing, feed intake was gradually increased until reaching *ad libitum* consumption. From day 11 of lactation, creep feed was offered to piglets *ad libitum*.

Blood sampling

The 36 sows were sampled for blood at day 108 of gestation (gestating sows) and after weaning (lactating sows). In addition, blood samples from the 48 selected piglets (two lightest and two heaviest birth weights in each litter) of the first batch of 12 sows were also obtained at weaning. Blood was collected by jugular venepuncture in non-heparinised tubes and in tubes with ethylenediaminetetraacetic acid (EDTA) for serum and plasma separation, respectively. Non-heparinised tubes were held at ambient temperature until centrifugation (3 000 rpm, 10 min), and EDTA samples were kept at 4 °C (maximum 120 min) until centrifugation (3 000 rpm, 10 min). Aliquots of serum for FA, and aliquots of plasma for oxylipins (in tubes containing 0.005 % butylated hydroxytoluene (Merck, Darmstadt, Germany) as antioxidant), Ig and cytokines were obtained and quickly stored at −80 °C (maximum 30 min from centrifugation to storage).

Quantitative analysis of fatty acids

Fat from serum of sows and piglets was extracted with chloroform (PanReac AppliChem, Barcelona, Spain) – methanol (Honeywell, Charlotte, NC, USA) according to Folch et al. (1957) and transmethylated with boron trifluoride (Sigma Aldrich, St. Louis, MO, USA) and methanolic potassium hydroxide 0.5 M (PanReac, Barcelona, Spain) according to Morrison and Smith (1964). Fatty acids were determined by gas chromatography (Agilent 6890N, Boston, MA, USA) according to the procedure previously described

in Llauradó-Calero et al. (2021). FAs were quantified from C12:0, and results were expressed as mg of FA per g of serum.

Metabolomic analysis of oxylipins

Fifty-three oxylipins were quantified from the plasma of sows and piglets. Preparation of samples was performed as previously described in Llauradó-Calero et al. (2021) for colostrum and milk samples but optimised for plasma samples. In brief, aliquots of 0.25 ml of plasma were mixed with 0.1 ml of internal standard mixture prepared in methanol (internal standard concentrations: Deuterated Primary COX and LOX LC-MS Mixture (10 μg/l), Deuterated Linoleic Acid Oxylipins LC-MS Mixture (5 μg/l), Leukotriene B4-d4 (50 μg/L), Lipoxin A4-d5 (50 μg/l), Arachidonic acid-d8 (1 mg/l), Resolvin D1-d5 (5 μg/l), 8-isoProstaglandin F2a-d4 (100 μg/l) (Cayman chemicals, Ann Arbor, MI, USA)). Thereafter, 0.5 ml of methanol (butylated hydroxytoluene 0.001 M) was added and samples were incubated for 30 min at −20 °C. After centrifugation, the supernatant was diluted with 4 ml of a 0.1 % of formic acid (Sigma Aldrich, St. Louis, MO, USA) in Milli-Q water (Millipore, Burlington, MA, USA). A clean-up using Oasis PRIME HLB cartridges (1 cc Vac Cartridge, 30 mg sorbent; Waters Corporation, Milford, MA, USA), the evaporation to dryness and the reconstitution of the samples were performed according to Ostermann (2017).

Oxylipin concentrations were determined using an ultra-HPLC 1290 Series coupled to a triple quadrupole MS 6490 series instrument (Agilent, Santa Clara, CA, USA) with an analytical column Eclipse XDB C18 1.8 μl (2.1 × 150 mm) (Agilent, Santa Clara, CA, USA). Gradient elution was performed using LC-MS water (Scharlab, Barcelona, Spain) (0.01 % acetic acid (Sigma Aldrich, St. Louis, MO, USA)) 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 (MRM). Identification with standards or tentative identification of oxylipins was previously described in Llauradó-Calero et al. (2021).

Immune indicators

The concentration of plasmatic IgG, IgA and IgM, and IL1β, IL6, IL10 and TNFα cytokines were quantitatively measured using sandwich ELISA kits as previously described in Llauradó-Calero et al. (2021).

Statistical analysis

The GLIMMIX procedure of SAS software (SAS/STAT 14.1; SAS Institute Inc., Cary, NC, USA) was used to perform the ANOVA. For gestating and lactating sows, the model included dietary treatment as fixed effect and batch as random effect, and for suckling piglets, the model included dietary treatment, birth body weight (**bBW**) category and the interaction between them as fixed effects and sow as random effect. 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 Kolmogorov-Smirnov test, and values were excluded if $P < 0.01$. Although a logarithmic transformation ($\log_{10}(X + 1)$) was performed to compare the oxylipin concentration between treatments, original means are presented in supplementary tables. Therefore, results are expressed as least squares means ± RMSE, except for oxylipins that are expressed as means ± RMSE (from transformed data). Differences were considered significant at $P < 0.05$, while those at $P < 0.1$ are reported as tendencies.

The PROC CORR procedure of SAS software was used to obtain the Pearson correlation coefficient between the concentrations of FA or oxylipins and the immune indicators that were significantly modified by the n-3 LCFA diet or by bBW. The significant correlation level was set at $P < 0.05$.

MetaboAnalyst 5.0 (https://www.metaboanalyst.ca, Alberta, CA, USA) was used to perform Principal Component Analysis (PCA) of FAs and oxylipins and the heatmap of oxylipins.

Results

Fatty acid profile

The changes caused by the inclusion of dietary fish oil on the FA profile of serum from gestating and lactating sows, and from piglets are shown in Tables 1 and 2, respectively.

Dietary n-3 LCFA did not change total saturated FAs in gestating and lactating sows but tended to decrease monounsaturated FAs ($P = 0.088$) in gestating sows. In gestating sows, the n-3 LCFA diet tended to increase myristic acid (C14:0) ($P = 0.093$), decreased heneicosylic acid (C21:0) ($P < 0.001$), and tended to decrease oleic acid (C18:1n-9 *cis* ($P = 0.061$)) compared to sows fed the control diet. However, in lactating sows, the only saturated or monounsaturated FA that was reduced by the n-3 LCFA diet was heneicosylic acid ($P = 0.002$). In terms of polyunsaturated FAs, n-3 LCFA diet increased total n-3 FAs, both at gestation and lactation ($P < 0.001$ and $P < 0.001$, respectively), EPA ($P < 0.001$ and $P < 0.001$, respectively), docosapentaenoic acid (C22:5n-3) ($P < 0.001$ and $P < 0.001$, respectively), and DHA ($P < 0.001$ and $P < 0.001$, respectively) compared to control diet. In contrast, total n-6 FA content did not differ between the two dietary treatments in gestating nor lactating sows. However, the n-3 LCFA diet reduced the concentrations of γ-linolenic acid (C18:3n-6 cis) ($P = 0.038$, $P < 0.001$, respectively), eicosadienoic acid (C20:2n-6 cis) ($P = 0.017$ and $P = 0.002$, respectively) and arachidonic acid (C20:4n-6) ($P < 0.001$ and $P < 0.001$, respectively) in gestating and lactating sows. Consequently, while total polyunsaturated FAs were not affected by dietary treatment, the n-6:n-3 ratio was reduced in the plasma of gestating ($P < 0.001$) and lactating ($P < 0.001$) sows that were fed the n-3 LCFA diet.

In piglets, no differences in the concentrations of total saturated and monounsaturated FAs were observed due to dietary treatment, bBW category or their interaction. However, regarding saturated FA, myristic acid (C14:0) was increased ($P = 0.003$) and lignoceric acid (C24:0) was decreased in the n-3 LCFA piglets ($P < 0.001$). In addition, heneicosylic acid (C21:0) was decreased in n-3 LCFA compared to control piglets ($P < 0.001$) and increased in LBW compared to high birth weight (**HBW**) piglets ($P = 0.039$). In terms of polyunsaturated FAs, on the one hand, total n-3 FAs were increased in the n-3 LCFA piglets ($P = 0.011$). Particularly, dietary n-3 LCFA increased α-linolenic acid (C18:3n-3 *cis*) ($P = 0.047$), EPA ($P < 0.001$), docosapentaenoic acid ($P < 0.001$) and DHA ($P < 0.001$), while no changes were observed in total n-6 FAs. On the other hand, the n-3 LCFA diet decreased γ-linolenic acid ($P = 0.009$), and an interaction between dietary treatment and body birth weight was observed for arachidonic acid ($P = 0.025$) where n-3 LCFA reduced the concentration of this FA only in the LBW piglets.

Principal component analysis shows the distribution of samples according to the type of sample (gestating sows, lactating sows or suckling piglets) and the dietary treatment (control or n-3 LCFA) (Fig. 1, A). EPA was the FA with the highest contribution in explaining the distribution of the principal component analysis. Another principal component analysis according to bBW category indicates that there is not a different distribution of FA profiles of suckling piglets in terms of this variable (Supplementary Fig. S1, A).

Table 1
Serum fatty acid profiles of gestating and lactating sows fed control or n-3 LCFA diets.[1,2]

	Gestating sows				Lactating sows			
	Control (n = 18)	n-3 LCFA (n = 18)	RMSE	*P*-value	Control (n = 18)	n-3 LCFA (n = 18)	RMSE	*P*-value
Fatty acid (mg FA/g serum)								
C12:0	0.033	0.037	<0.01	0.572	0.028	0.028	<0.01	0.996
C14:0	0.027	0.034	<0.01	0.093	0.002	0.003	<0.01	0.645
C16:0	0.75	0.71	0.03	0.455	0.65	0.65	0.02	0.956
C16:1	0.042	0.046	<0.01	0.499	0.044	0.043	<0.01	0.862
C18:0	0.57	0.51	0.02	0.133	0.54	0.52	0.01	0.512
C18:1n-9 *cis*	0.98	0.78	0.02	0.061	0.91	0.85	0.08	0.492
C18:1n-7	0.063	0.067	<0.01	0.567	0.078	0.074	<0.01	0.520
C18:2n-6 *cis*	1.04	1.11	0.11	0.567	1.02	0.89	0.11	0.285
C18:3n-6 *cis*	0.014	0.007	<0.01	0.038	0.026	0.022	<0.01	<0.001
C18:3n-3 *cis*	0.052	0.056	<0.01	0.384	0.036	0.039	<0.01	0.362
C20:2n-6 *cis*	0.015	0.011	<0.01	0.017	0.015	0.010	<0.01	0.002
C21:0	0.030	0.007	<0.01	<0.001	0.025	0.013	<0.01	0.002
C20:3n-6	0.012	0.013	<0.01	0.673	0.012	0.010	<0.01	0.431
C20:4n-6	0.26	0.12	<0.01	<0.001	0.27	0.18	<0.01	<0.001
C20:5n-3	0.003	0.16	<0.01	<0.001	0.001	0.14	<0.01	<0.001
C24:0	0.022	0.019	<0.01	0.144	0.018	0.018	<0.01	0.610
C22:5n-3	0.051	0.10	<0.01	<0.001	0.037	0.064	<0.01	<0.001
C22:6n-3	0.019	0.13	<0.01	<0.001	0.012	0.084	<0.01	<0.001
Minor FA[3]	0.27	0.25	<0.01	0.206	0.27	0.30	<0.01	0.118
SFA	1.52	1.40	0.11	0.331	1.38	1.37	0.07	0.884
MUFA	1.17	0.97	0.12	0.088	1.11	1.05	0.09	0.536
PUFA	1.56	1.77	0.21	0.180	1.50	1.52	0.21	0.898
n-3	0.19	0.48	<0.01	<0.001	0.14	0.38	<0.01	<0.001
n-6	1.37	1.29	0.16	0.533	1.36	1.15	0.16	0.113
n-6:n-3	7.51	2.68	2.21	<0.001	9.66	3.01	1.28	<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: C13:0, C14:1n-9 *cis*, C15:0, C15:1, C17:0, C17:1, C18:1n-9 *trans*, C18:1n-11 *cis*, C18:2n-6 *trans*, C19:0, C18:4n-3, C20:0, C20:1n-9 *cis*, C20:3n-3 *cis*, C22:0, C22:1, C22:2n-6 *cis*, C23:0, C22:4n-6, C22:3n-3, and C24:1n-9 *cis*. C13:0 and C14:1n-9 *cis* have not been detected in plasma from lactating and gestating sows, respectively.

Oxylipin profile

Dietary n-3 LCFA inclusion modified the plasma concentrations of 25 oxylipins in gestating sows and 28 oxylipins in lactating sows. Concretely, differences between treatments are mainly due to increases in the concentrations of oxylipins derived from EPA and DHA in the sows that were fed the fish oil diet (Supplementary Table S1 and S2). In gestating sows, the EPA-derived oxylipins that were increased by n-3 LCFA were 5(s)-, 8-, 9-, 11-, 12(s)-, 15(s)- and 18-hydroxy-EPA ($P < 0.001$). In terms of DHA-derived oxylipins, n-3 LCFA increased 4-, 7-, 8-, 10-, 11-, 13-, 14-, 16-, 17- and 20-hydroxy-DHA ($P < 0.001$). In addition, fish oil also modified some oxylipins that are derived from other LCFAs. Concretely, n-3 LCFA tended to increase 15(16)-epoxy-octadecadienoic acid ($P = 0.096$) that is derived from α-linolenic acid and 15,16-dihydroxy-octadecenoic acid ($P = 0.069$) that is derived from linoleic acid. It also increased 15(R)-Lipoxin A4/Lipoxin A4 ($P = 0.012$), decreased 8,9-, 11(12)- and 14,15-dihydroxy-eicosatrienoic acid ($P < 0.001$, $P = 0.014$ and $P = 0.010$, respectively), 20-hydroxy-eicosatetraenoic acid ($P = 0.008$) and tended to reduce 11,12-epoxy-eicosatrienoic acid ($P = 0.076$) that are derived from arachidonic acid. In lactating sows, n-3 LCFA also increased the EPA-derived oxylipins 5(s)-, 8-, 9-, 11-, 12(s)-, 15(s)- and 18-hydroxy-EPA ($P < 0.001$), and the DHA-derived oxylipins 4-, 7-, 8-, 10-, 11-, 13-, 14-, 16-, 17- and 20-hydroxy-DHA ($P < 0.001$). In addition to these modifications, lactating sows fed n-3 LCFA presented increased concentrations of 15 (R)-Lipoxin A4/Lipoxin A4 ($P < 0.001$) and 8-hydroxy-eicosatetraenoic acid ($P = 0.004$), which are derived from arachidonic acid. They also had decreased concentrations (or tendencies to decrease) of 9(10)-epoxy-octadecadienoic acid ($P = 0.072$) and 9 (s)-hydroxy-octadecatrienoic acid ($P = 0.044$), which are derived from α-linolenic acid; 9(10)- and 12(13)-epoxy-octadecenoic acid ($P = 0.012$ and $P = 0.071$, respectively), derived from linoleic acid; and 8,9-, 11(12)- and 14,15-dihydroxy-eicosatrienoic acid ($P = 0.021$, $P = 0.043$ and $P = 0.021$, respectively), 12(s)-hydroxy-eicosatetraenoic acid ($P = 0.097$) and 14,15-epoxy-eicosatrienoic acid ($P = 0.014$) derived from arachidonic acid.

The piglets' plasma oxylipin concentrations according to dietary treatment and birth BW category are reported in Supplementary Table S3. The inclusion of fish oil in the diet modified a total of 26 oxylipins. Concretely, dietary n-3 LCFA increased the EPA-derived oxylipins 5(s)-, 8-, 9-, 11-, 12(s)-, 15(s)- and 18-hydroxy-EPA ($P < 0.001$); the DHA-derived oxylipins 4-, 7-, 8-, 10-, 11-, 13-, 14-, 16-, 17- and 20-hydroxy-DHA ($P < 0.001$); the α-linolenic acid-derived oxylipin 15(16)-epoxy-octadecadienoic acid ($P = 0.014$); and the linoleic acid-derived oxylipin 9,10-dihydroxy-octadecadienoic acid ($P = 0.036$). On the other hand, n-3 LCFA also decreased (or tended to decrease) the DHA-derived oxylipin 13,14-dihydoxy-docosapentaenoic acid ($P = 0.006$); and the arachidonic acid-derived oxylipin thromboxane B2 ($P = 0.052$), 8,9- and 11 (12)-dihydroxy-eicosatrienoic acid ($P = 0.051$ and $P = 0.085$, respectively), 8- and 20-hydroxy-eicosatetraenoic acid ($P = 0.018$ and $P = 0.021$, respectively), and 11,12-epoxy-eicosatrienoic acid ($P = 0.099$). Moreover, the plasma concentrations of 17 oxylipins

Table 2
Serum fatty acid profiles of suckling piglets from sows fed control or n-3 LCFA diets, born with low or high BW.[1,2,3]

	Suckling piglets				RMSE	*P*-value MDiet	*P*-value bBW
	MDiet		bBW				
	Control (n = 28)	n-3 LCFA (n = 20)	HBW (n = 24)	LBW (n = 24)			
Fatty acid (mg FA/g serum)							
C12:0	0.018	0.019	0.019	0.018	<0.01	0.662	0.883
C14:0	0.061	0.086	0.071	0.076	<0.01	0.003	0.560
C16:0	1.68	1.90	1.73	1.85	0.18	0.114	0.354
C16:1	0.25	0.30	0.27	0.28	0.01	0.195	0.833
C18:0	0.76	0.79	0.74	0.80	0.02	0.742	0.127
C18:1n-9 *cis*	1.31	1.35	1.26	1.40	0.24	0.819	0.332
C18:1n-7	0.14	0.14	0.14	0.15	<0.01	0.906	0.438
C18:2n-6 *cis*	1.54	1.51	1.55	1.51	0.24	0.835	0.766
C18:3n-6 *cis*	0.035	0.028	0.031	0.032	<0.01	0.009	0.794
C18:3n-3 *cis*	0.030	0.040	0.035	0.036	<0.01	0.047	0.856
C20:2n-6 *cis*	0.031	0.025	0.026	0.030	<0.01	0.128	0.151
C21:0	0.027	0.013	0.018	0.021	<0.01	<0.001	0.039
C20:3n-6	0.025	0.025	0.023	0.027	<0.01	0.827	0.174
C20:4n-6*	0.52	0.33	0.42	0.44	<0.01	0.006	0.507
C20:5n-3	0.009	0.18	0.10	0.088	<0.01	<0.001	0.375
C24:0	0.034	0.017	0.025	0.026	<0.01	<0.001	0.912
C22:5n-3	0.065	0.11	0.083	0.088	<0.01	<0.001	0.550
C22:6n-3	0.20	0.46	0.26	0.40	0.09	<0.001	0.171
Minor FA[4]	0.31	0.25	0.28	0.28	<0.01	0.328	0.908
SFA	2.65	2.91	2.68	2.88	0.33	0.268	0.236
MUFA	1.87	1.91	1.82	1.96	0.37	0.819	0.448
PUFA	2.54	2.75	2.58	2.71	0.65	0.454	0.323
n-3	0.37	0.81	0.52	0.66	0.43	0.011	0.268
n-6	2.17	1.94	2.06	2.05	0.35	0.240	0.929
n-6:n-3	7.72	2.67	5.52	4.87	1.23	<0.001	0.767

bBW, birth weight; FA, fatty acid; HBW, high birth weight piglets; LBW, low birth weight piglets; LCFA, long-chain fatty acid; MDiet, maternal diet; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
*P-value of the interaction MDiet*bBW for C20:4n-6 was $P = 0.025$ where the concentrations in mg FA/g serum were 0.56^{a} for control-LBW, 0.48^{b} for control-HBW, 0.31^{c} for n-3 LCFA-LBW, and 0.36^{bc} for n-3 LCFA-HBW.
[1] Values are least squares means ± RMSE.
[2] FA quantification results are reported from C12:0.
[3] The *P*-value of the interaction MDiet*bBW is not reported in the table since only significant *P*-values were observed for C20:4n-6 concentration.
[4] Minor FAs include: C14:1n-9 *cis*, C15:0, C15:1, C17:0, C17:1, C18:1n-9 *trans*, C18:1n-11 *cis*, C18:2n-6 *trans*, C19:0, C18:4n-3, C20:0, C20:1n-9 *cis*, C22:0, C22:1, C22:2n-6 *cis*, C23:0, C22:4n-6, C22:3n-3, and C24:1n-9 *cis*. C13:0 and C20:3n-3 *cis* have not been detected.

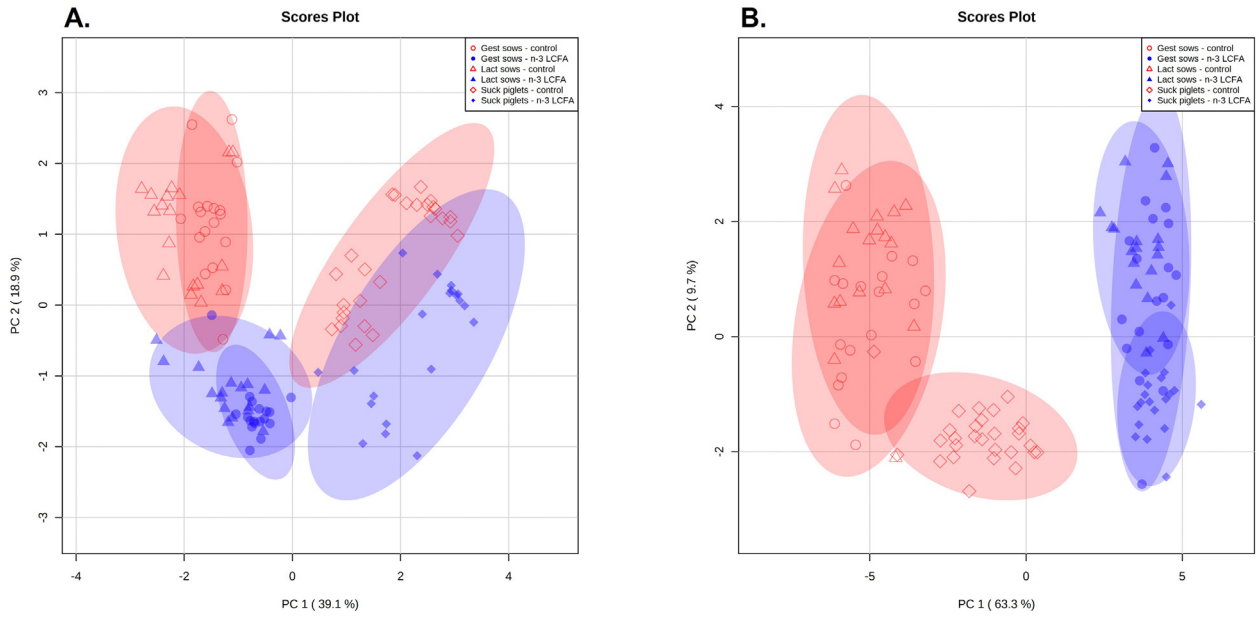

Fig. 1. Principal component analysis 2 dimensions score plot showing the effect of dietary fish oil on fatty acids (FAs) in serum (A) and oxylipins in plasma (B) of gestating and lactating sows and suckling piglets. Values are means of 18 samples per treatment in gestating and lactating sows, and means of 28 control and 20 n-3 LCFA samples in suckling piglets. Gest sows, gestating sows; Lact sows, lactating sows; LCFA, long-chain fatty acid; Suck piglets, suckling piglets.

also differed between LBW and HBW piglets. The effect of bBW was observed mainly for oxylipins that were derived from n-6 FAs. Compared to HBW piglets, the LBW piglets had (or tended to have) higher concentrations of 9,10- and 15,16-dihydroxy-octadecadienoic acid ($P = 0.072$ and $P = 0.048$, respectively), 9(10)- and 12(13)-epoxy-octadecenoic acid ($P = 0.087$ and $P = 0.082$, respectively), 9(s)- and 13(s)-hydroxy-octadecadienoic ($P = 0.006$ and $P = 0.050$, respectively), and 9- and 13-oxo-octadecadienoic ($P = 0.047$ and $P = 0.056$, respectively) that are derived from linoleic acid; and of 5,6-dihydroxy-eicosatrienoic acid ($P = 0.015$), and 5(s)-, 11-, 12(s)-, 15(s)- and 20-hydroxy-eicosatetraenoic acid ($P = 0.007$, $P = 0.025$, $P < 0.001$, $P = 0.017$ and $P = 0.042$, respectively) that are derived from arachidonic acid. In addition, 5(s) and 15(s)-hydroxy-EPA ($P = 0.053$ and $P = 0.003$, respectively) and 13-hydroxy-DHA ($P = 0.059$), which are derived from n-3 LCFA were also increased in the LBW piglets.

The 2D plots obtained through principal component analysis shows clearly differentiated plasma oxylipin profiles for gestating and lactating sows and piglets (Fig. 1, B). However, the differences observed between sample types could not be explained by changes in any particular oxylipin and they were due to overall changes in the whole oxylipin profile. The heatmap represented in Fig. 2 illustrates how the plasma concentrations of n-3 FA-derived oxylipins in gestating and lactating sows and piglets were clearly increased in the fish oil group. Moreover, it can also be observed that, independently of the dietary treatment, sows presented higher concentrations of oxylipins derived from α-linolenic acid and linolenic acid, while piglets presented higher concentrations of arachidonic acid-derived metabolites. Finally, schematic overviews of the oxylipins from different FA precursors that were modified by dietary n-3 LCFA inclusion or by bBW are summarised in Supplementary Figs. S2 and S3.

Immunological analysis

The plasma concentrations of Ig and cytokines in gestating and lactating sows are shown in Table 3. Dietary fish oil increased IgM in both gestating and lactating sows ($P = 0.014$ and $P = 0.008$, respectively) and IL6 in lactating sows.

The plasma immune indicators in piglets are reported in Table 4. An interaction between dietary treatment and bBW was observed for IgA ($P = 0.043$). The plasmatic concentration of IgA was highest in the HBW piglets of the control group, which differed from those of control-LBW and n-3 LCFA-HBW piglets. Regarding the cytokines, both dietary treatment and bBW group had significant effects on IL1β concentrations, which were higher in the n-3 LCFA than the control piglets ($P = 0.018$) and in the HBW than the LBW piglets ($P = 0.002$).

Correlations between fatty acids or oxylipins and immune indicators

In gestating sows, the IgM concentration was negatively correlated with eicosadienoic acid ($r = -0.36$, $P = 0.030$; Fig. 3, A) and positively correlated with 5(s)-, 9-, 11- and 12(s) hydroxy-EPA ($r = 0.41$, $P = 0.013$; $r = 0.38$, $P = 0.030$; $r = 0.37$, $P = 0.027$ and $r = 0.36$, $P = 0.030$, respectively), and 8- and 11-hydroxy-DHA ($r = 0.34$, $P = 0.042$ and $r = 0.35$, $P = 0.039$, respectively; Fig. 3, B).

In lactating sows, the concentrations of IgM and IL6 were negatively correlated with those of the n-6 family FAs γ-linolenic acid ($r = -0.42$, $P = 0.028$ and $r = -0.54$, $P = 0.004$, respectively) and arachidonic acid ($r = -0.44$, $P = 0.021$ and $r = -0.39$, $P = 0.044$, respectively) (Fig. 3, C). Moreover, IgM was positively correlated with the arachidonic acid-derived oxylipins 15(R)-Lipoxin A4/Lipoxin A4 ($r = 0.39$, $P = 0.042$) and 8-hydroxy-eicosatetraenoic acid ($r = 0.41$, $P = 0.035$), and all the detected hydroxy-EPAs and hydroxy-DHAs (all $r \geq 0.39$, $P \leq 0.017$), particularly with 14-hydroxy-DHA for which a relatively high positive correlation was observed ($r = 0.64$, $P < 0.001$). IL6 was also positively correlated with most of the hydroxy-EPA and hydroxy-DHA oxylipins (all $r \geq 0.39$, $P \leq 0.046$; Fig. 3, D).

In piglets, IL1β was positively correlated with the n-3 family FAs: α-linolenic acid ($r = 0.34$, $P = 0.021$), EPA ($r = 0.57$, $P < 0.001$) and docosapentaenoic ($r = 0.49$, $P < 0.001$) acid (Fig. 3, E). Moreover, IL1β was also positively correlated with 15(16)-epoxy-octadecadienoic acid which is derived from α-linolenic acid ($r = 0.38$, $P = 0.007$); 9,10-dihydroxy-octadecadienoic acid ($r = 0.32$, $P = 0.026$) and 9(s)-hydroxy-octadecadienoic acid ($r = 0.45$, $P = 0.001$) derived from linoleic acid; all hydroxy-EPA detected, derived from EPA; and most hydroxy-DHA detected, derived from DHA (all $r \geq 0.49$, $P < 0.001$; Fig. 3, F). Among the positive correlations observed, those with 4- and 14-hydroxy-DHA stand out ($r = 0.70$, $P < 0.001$ and $r = 0.72$, $P < 0.001$, respectively). Finally, a negative correlation was also observed between IL1β and the DHA-derived oxylipin 13,14-dihydroxy-docosapentaenoic acid ($r = -0.44$, $P = 0.002$).

Discussion

Piglets are born with low energy stores and without an effective immune system (Le Dividich et al., 2005), and recently, this has been further aggravated by the increasing proportion of LBW piglets born from hyperprolific sows, which makes them even more vulnerable (Farmer and Edwards, 2022). Changing the source of fat in sow (and the consequent changes in colostrum and milk composition) and piglet diets could have a positive impact on the physiology of newborn piglets, particularly as it has been described that they are able to digest more than 90 % of the lipids present in colostrum and milk (Azain, 2001). In the current study, the inclusion of fish oil in sow diets increased the total amount of n-3 polyunsaturated FAs in serum from gestating and lactating sows mainly by increasing the amounts of EPA, docosapentaenoic acid and DHA. Moreover, the n-3 LCFA diet also decreased the concentration of some minor saturated or n-6 polyunsaturated FAs such as heneicosylic acid, γ-linolenic acid, eicosadienoic acid and arachidonic acid, without modifying the amounts of total saturated or n-6 polyunsaturated FAs. In addition, the changes in the FA profile of piglets' serum observed with the n-3 LCFA-rich diet are in line with those observed in gestating and lactating sows and with those recently reported by our research group in the colostrum and milk of the same sows (Llauradó-Calero et al., 2021). Furthermore, the changes observed in the serum of suckling piglets were also in good agreement with studies that evaluated different dietary n-6: n-3 ratios in serum of presuckling piglets (Eastwood et al., 2014) and plasma of piglets at day 21 of lactation (Yao et al., 2012).

It is well established that polyunsaturated FAs can influence the coordination of a balanced inflammatory response. One of the mechanisms of action could be through oxidation processes via enzymatic or non-enzymatic pathways and the consequent formation of oxylipins (Calder, 2010). Each oxygenated FAderivative can exert a proinflammatory and/or anti-inflammatory activity (Gabbs et al., 2015), which could influence not only the immune system of sows, but also that of piglets, which may be particularly relevant as they are born with an absolute absence of immune protection (Le Dividich et al., 2005). Moreover, oxylipins derived from n-3 FAs tend to have weak proinflammatory, anti-inflammatory or inflammation resolving properties, whereas those derived from n-6 FA are usually associated with proinflammatory activities (Calder, 2010). The enhanced concentrations of EPA and DHA in the serum of gestating and lactating sows and of piglets resulted in increases of their hydroxy-derivatives in plasma, which are mainly generated through the lipoxygenase enzymatic pathway, in some speci-

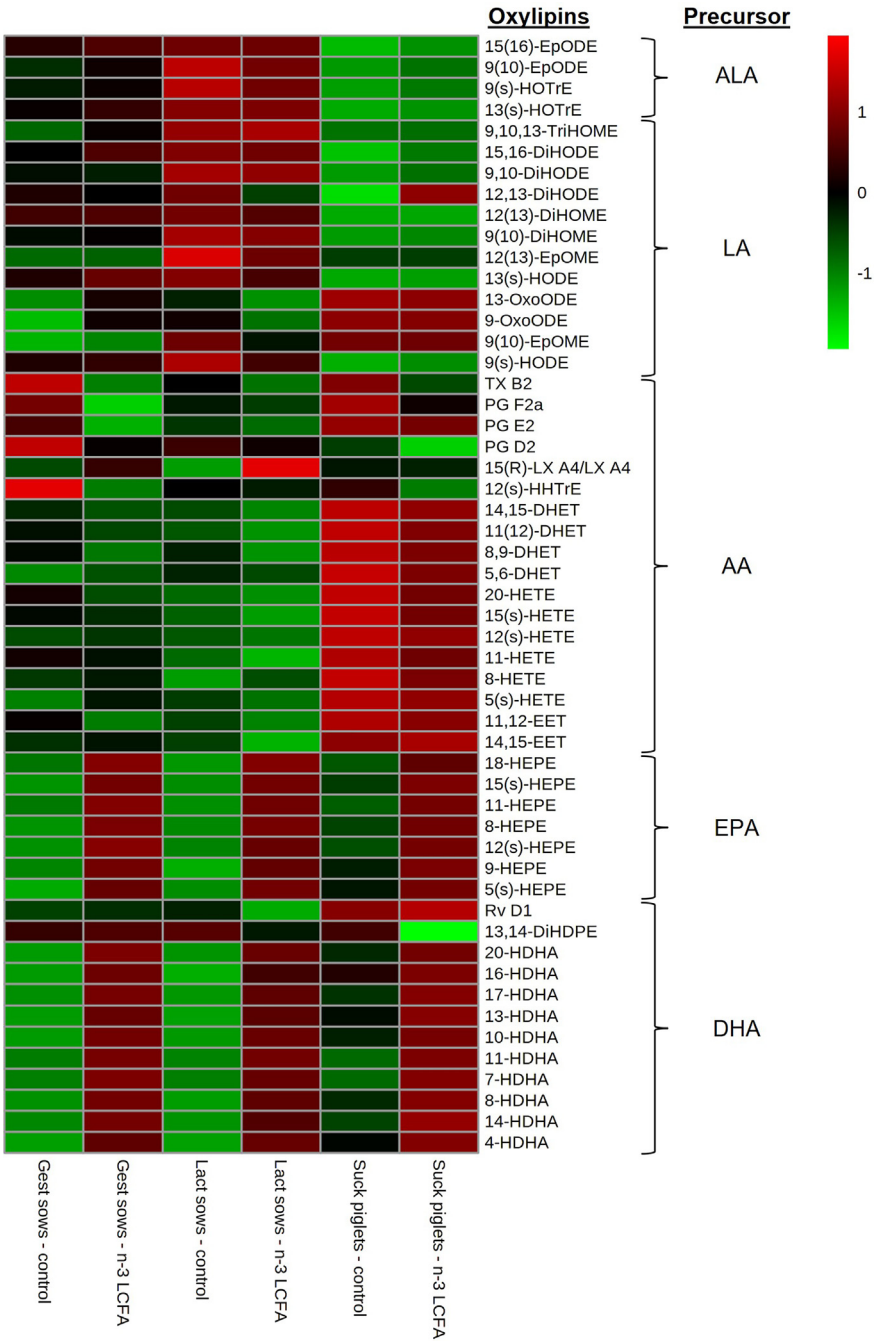

Fig. 2. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)-derived oxylipins are increased in plasma of gestating and lactating fish oil-fed sows, and suckling piglets from fish oil-fed sows. Each coloured cell on the map corresponds to a concentration value being green lower concentrations and red higher concentrations. Values are means of 18 samples per treatment in gestating and lactating sows, and means of 28 control and 20 n-3 LCFA samples in suckling piglets. AA, arachidonic acid; ALA, α-linolenic acid; DiHDPE, dihydroxy-docosapentaenoic 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; Gest sows, gestating sows; 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; Lact sows, lactating sows; LCFA, long-chain fatty acid; LT, Leukotriene; LX, Lipoxin; OxoODE, oxo-octadecadienoic acid; PG, Prostaglandin; Suck piglets, suckling piglets; TriHOME, trihydroxy-octadecenoic acid; TX, Thromboxane.

fic cases through the cytochrome P450 enzymatic pathway, or also via non-enzymatic pathways (Astarita et al., 2015). Although, for most of these oxylipins, precise functions have only been described in specific murine or human cell lines, some of them, such as 18-hydroxy-EPA, 13-hydroxy-DHA or 17-hydroxy-DHA, are related with an anti-inflammatory role and with the inhibition of the production of proinflammatory cytokines such as TNFα (Gabbs et al., 2015). In addition, these results are also in line with those reported by our group in colostrum and milk (Llauradó-Calero et al., 2021), which suggests a direct influence of including fish oil in the sow diet on the oxylipin profile of the suckling piglets by increasing the concentration of oxylipins with an anti-inflammatory role. In addition, the n-3 LCFA diet also changed the concentration of oxylipins derived from other FAs. In the review of Shearer and Walker (2018), it is described that a major increase in circulating n-3 oxylipins commonly leads to a decrease in n-6 FA-derived

Table 3
Plasma immune indicators in gestating and lactating sows fed control or n-3 LCFA diets[1].

	Gestating sows				Lactating sows[2]			
	Control (n = 18)	n-3 LCFA (n = 17)	RMSE	*P*-value	Control (n = 13)	n-3 LCFA (n = 14)	RMSE	*P*-value
Immunoglobulins (mg/ml)								
IgG	130	159	52.2	0.396	5.27	5.08	1.51	0.864
IgA	2.55	3.14	0.54	0.108	1.44	1.62	0.49	0.614
IgM	3.95	4.86	0.53	0.014	4.43	6.34	0.91	0.008
Cytokines (ng/ml)								
IL1β	17.4	26.0	12.5	0.314	145	204	80.8	0.322
IL6	34.8	83.1	45.7	0.116	136	421	147	0.012
IL10	1.89	11.2	10.6	0.198	0.11	0.68	0.64	0.240
TNFα	1.01	1.44	0.84	0.444	1.40	0.91	1.20	0.579

IgA, immunoglobulin A; IgG, Immunoglobulin G; IgM, immunoglobulin M; IL1β, interleukin 1β; IL6, interleukin 6; IL10, interleukin 10; TNFα, tumour necrosis factor α; LCFA, long-chain fatty acid.
[1] Values are least squares means ± RMSE.
[2] Samples from the second batch of sows (5 from control diet and 4 from n-3 LCFA diet) could not be analysed due to a conservation issue.

Table 4
Plasma immune indicators of suckling piglets from sows fed control or n-3 LCFA diet, born with low or high BW.[1,2]

	Suckling piglets				RMSE	*P*-value Diet	*P*-value bBW
	MDiet		bBW				
	Control (n = 28)	n-3 LCFA (n = 20)	HBW (n = 24)	LBW (n = 24)			
Immunoglobulins (mg/ml)							
IgG	7.46	12.0	10.6	8.88	1.80	0.110	0.112
IgA*	0.42	0.37	0.41	0.38	0.07	0.373	0.431
IgM	0.62	0.68	0.67	0.63	0.31	0.723	0.622
Cytokines (ng/ml)							
IL1β	6.08	22.1	15.1	13.1	2.11	0.018	0.002
IL6	13.5	17.0	17.7	12.8	19.0	0.802	0.389
IL10	0.24	0.54	0.39	0.39	0.32	0.132	0.897
TNFα	0.22	0.29	0.28	0.23	0.18	0.580	0.375

bBW, birth weight; HBW, high birth weight piglets; IgA, immunoglobulin A; IgG, Immunoglobulin G; IgM, immunoglobulin M; IL1β, interleukin 1β; IL6, interleukin 6; IL10, interleukin 10; LBW, low birth weight piglets; LCFA, long-chain fatty acid; MDiet, maternal diet; TNFα, tumour necrosis factor α.
*P-value of the interaction MDiet*bBW for IgA was $P = 0.043$ where the concentrations in mg/ml were 0.37^{b} for control-LBW, 0.48^{a} for control-HBW, 0.40^{ab} for n-3 LCFA-LBW, and 0.35^{b} for n-3 LCFA-HBW.
[1] Values are least squares means ± RMSE.
[2] The *P*-value of the interaction MDiet*bBW is not reported in the table since only significant *P*-values were observed for IgA and n-3 concentrations.

oxylipins, which was also observed in this study, particularly for those derived from arachidonic acid. However, Shearer and Walker (2018) also report that in some cases, oxylipins from n-6 FAs can be increased with n-3 FA supplementation, which could explain the increases observed for certain oxylipins derived from linoleic acid or arachidonic acid. Among the changes caused by n-3 LCFA diet in the piglets' plasma concentrations of n-6 FA-derived oxylipins, the reduced levels of 20-hydroxy-eicosatetraenoic acid stand out, and this oxylipin has been described to stimulate proinflammatory cytokine production in human endothelial cells (Gabbs et al., 2015). In addition, two final oxidation products were modified by fish oil: concretely, lipoxin A4 was increased in gestating and lactating sows and thromboxane B2 tended to decrease in suckling piglets. On the one hand, lipoxin A4 is the main physiological lipoxin during inflammation in mammalian systems and has been proven to have a powerful anti-inflammatory role under many pathological conditions that trigger inflammation (Shi et al., 2017). In contrast, thromboxane B2 is related with vascular resistance, vasoconstriction activity and platelet aggregation (Gabbs et al., 2015).

To our knowledge, this is the first study assessing the effect of bBW on plasma oxylipin concentrations. It was observed that some oxylipins derived from the n-6 FA linoleic acid and arachidonic acid were increased in LBW piglets. However, for most of them, a well-defined function is not known yet, which makes it difficult to understand the role that bBW may have on oxylipin-mediated activities.

Moreover, both, n-3 FAs and their oxygenated derivates, can influence the production of certain Ig (Mitre et al., 2005; Calder, 2010; Yao et al., 2012). In the current study, n-3 LCFA increased IgM concentrations in the blood of gestating and lactating sows, although no changes in IgM concentration were reported in the colostrum and milk of the same sows (Llauradó-Calero et al., 2021). Other studies evaluating the effect of n-3 FAs in sow diets describe changes in the concentration of immunoglobulins in plasma of suckling piglets at different times during lactation (Mitre et al., 2005; Leonard et al., 2010; Yao et al., 2012), yet immunoglobulin concentrations in suckling piglets were not affected in the current study. However, in all the mentioned reports, the effect of increasing the concentration of the different types of immunoglobulins fades as lactation progresses. Leonard et al. (2010) observed differences in IgM concentration in the serum of piglets from sows that were supplemented with sharkoil at the beginning of lactation but not at the time of weaning, which could explain why we did not observe any changes at the end of lactation in our study. In addition, we showed that IgM was positively correlated with the increase of oxylipins derived from n-3 FAs, but negatively correlated with FAs from the n-6 family such

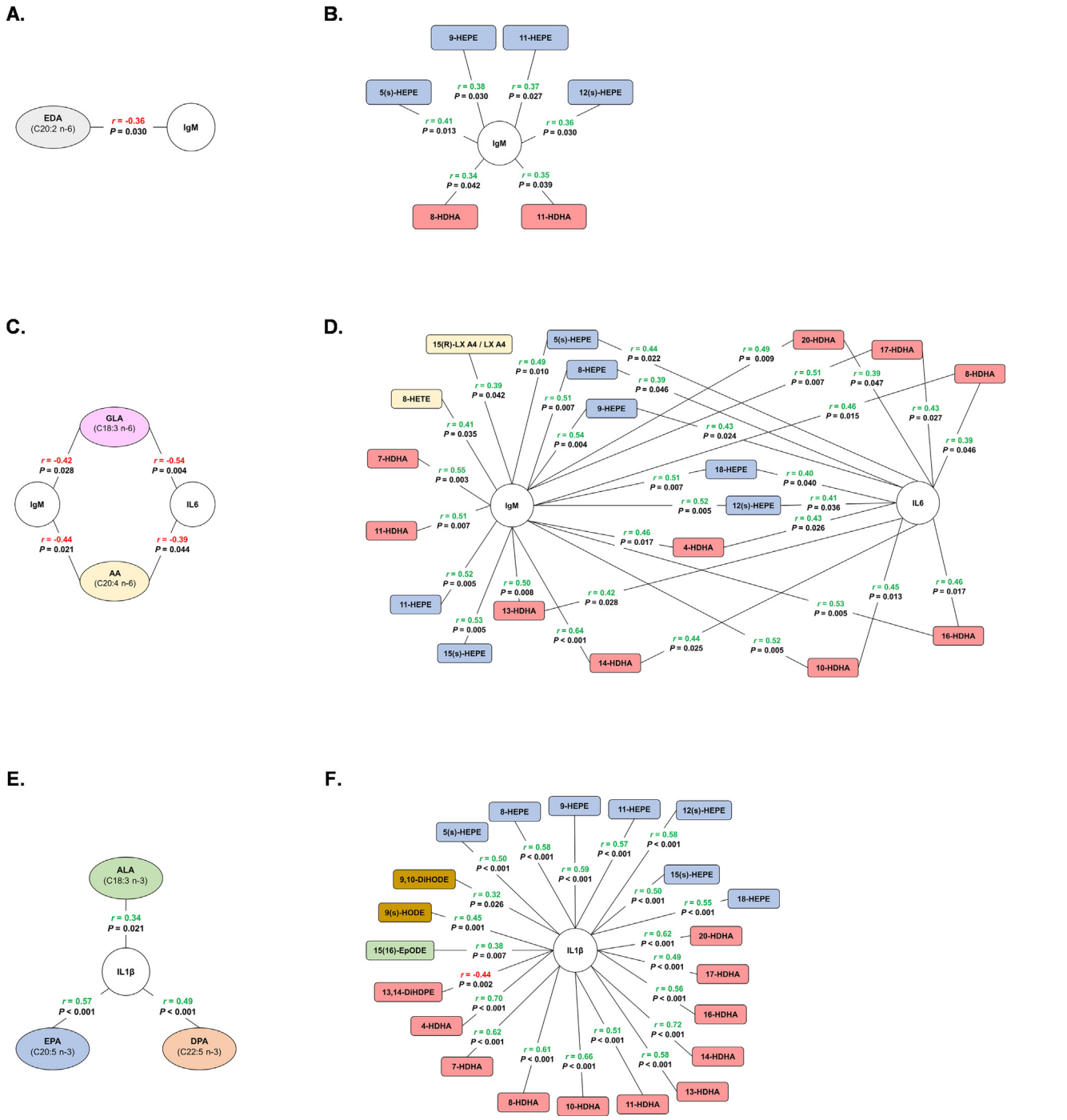

Fig. 3. Correlations between FA and oxylipins and immune indicators modified by n-3 LCFA diet for gestating (A and B) and lactating sows (C and D), and suckling piglets (E and F). Pearson correlation coefficient (r) in red and in green indicates negative and positive correlation, respectively. Significant correlation level was set at $P < 0.05$. Fatty acids and their derived oxylipins are represented by the same colour. AA, arachidonic acid; ALA, α-linolenic acid; DiHDPE, dihydroxy-docosapentaenoic acid; DiHODE, dihydroxy-octadecadienoic acid; EDA, eicosadienoic acid; EPA, eicosapentaenoic acid; EpODE, epoxy-octadecadienoic acid; DPA, docosapentaenoic acid; GLA, γ-linolenic acid; HDHA, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HODE, hydroxy-octadecadienoic acid; IgM, immunoglobulin M; IL1β, interleukin 1β; IL6, interleukin 6; LX, Lipoxin.

as eicosadienoic acid, γ-linolenic acid and arachidonic acid. In terms of cytokine production, increases in the plasmatic concentrations of IL6 in lactating sows and IL1β in suckling piglets were observed with the n-3 LCFA diet, whereas cytokine IL1β was also higher in HBW than LBW piglets. On the one hand, IL6 is a soluble mediator with a pleiotropic effect on inflammation and immune response (Tanaka et al., 2014), and the role that its increase may play, without modifications on other cytokines concentrations, is not clear. Furthermore, in line with our results for plasmatic IgM, IL6 concentration was negatively correlated with n-6 FAs and positively correlated with most of the n-3 FA-derived oxylipins. On the other hand, IL1β is considered to be one of the major proinflammatory cytokines (Nordgreen et al., 2020). The observed increase in the concentration of IL1β has also been reported by Yao et al., 2012 in suckling piglets from sows that were fed a diet with an n-6:n-3 ratio of 3:1, which is similar to the ratio used in this exper-

iment, in comparison to ratios of 9:1 or 13:1. Therefore, and considering that the inclusion of n-3 LCFA in the diets resulted in increases of oxylipins with anti-inflammatory roles, and that the concentration of IL1β was positively correlated with both, the n-3 FAs and their oxygenated derivatives, future studies evaluating a wider range of cytokines and their relationship with n-3 FAs in piglets may help to better understand the results obtained in this study.

Conclusion

This study provides a picture of precursors, intermediate molecules, and final mediators in serum and plasma of gestating sows, lactating sows and suckling piglets that had been fed diets with or without supplementation with n-3 LCFA, which complement our previous study in which these parameters were described in colostrum and milk. It can be concluded that the dietary inclusion of fish oil rich in EPA and DHA offered to sows during gestation and lactation increases the blood concentrations of EPA, DHA and their oxygenated derivatives with anti-inflammatory role in sows and suckling piglets. In addition, dietary n-3 LCFA also had an impact on immune indicators such as IgM, which was increased in gestating and lactating sows, and IL6 and IL1β that were increased in lactating sows and suckling piglets, respectively. In addition, although the bBW of the piglets had little effect on FA composition, it was observed that LBW piglets had higher concentrations of oxylipins derived from n-6 FA (i.e. LA or AA) and reduced IL1β compared to HBW piglets. Overall, further research is required to confirm the feasibility of improving piglets' vitality through the inclusion of n-3 LCFA in sow and piglet diets.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100634.

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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