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Effect of fatty acid composition of the sow diet on the innate and adaptive immunity of the piglets after weaning

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ABSTRACT

This study investigated whether the immunocompetence of piglets at weaning was modulated by including different sources of n-3 polyunsaturated fatty acids (PUFA) in the maternal diet. From day 73 of gestation until weaning at 4 weeks, 32 pregnant sows were fed a palm oil-based diet (control group) or a diet including 1% linseed oil (C18:3n-3), 1% echium oil (C18:3n-3, C18:4n-3, C18:3n-6) or 1% fish oil (C20:5n-3, C22:6n-3). It was hypothesized that each diet would differently affect immune function through effects such as specific eicosanoid production. Piglets were fed a conventional diet without added n-3 PUFA from weaning until day 35 post-weaning. At weaning and 21 days post-weaning, four piglets per litter were immunized with bovine thyroglobulin. Blood samples were taken from weaning until day 35 post-weaning to determine thyroglobulin-specific antibodies, serum amyloid A (SAA) concentration and fatty acid composition. The fatty acid composition of the maternal diets was reflected in the plasma and red blood cells of the weaned piglets. The onset of the thyroglobulin-specific IgM response differed between dietary groups, with a delay in response for piglets from sows fed the fish oil diet. No significant dietary effects were observed on the thyroglobulin-specific IgG and IgA titres or on SAA concentrations in the piglet serum. Including n-3 PUFA in the maternal diet at the concentrations used in the present study had no major effects on the adaptive and innate immunity of the piglets after weaning.

Introduction

Environmental exposures early in life are important for health and disease prevention. Nutrition during pregnancy is a source of early exposure that might have a fundamental influence on fetal development (Prescott and Dunstan, 2007; Calder et al., 2010). Dietary fatty acids (FA) are of particular interest, as they are incorporated into the membranes of all cells including those of the immune system. Altered FA composition of the immune cells can influence their function through several mechanisms including changing membrane fluidity, altering their affinity as substrates for enzymes involved in cell signalling pathways, and by modifying the amount and type of eicosanoids produced (Calder and Grimble, 2002).

Eicosanoids are a group of chemical messengers that are synthesized from polyunsaturated fatty acids (PUFA), in particular from arachidonic acid (C20:4n-6, ARA), but also from dihomo-γ-linolenic acid (C20:3n-6, DGLA) and eicosapentaenoic acid (C20:5n-3, EPA). These eicosanoids exert pro- as well as anti-inflammatory effects, and their overall effect depends on their concentrations and the balance between their precursor FA in the cell membranes (Calder, 2001). In addition, EPA and docosahexaenoic acid (C22:6n-3, DHA) can give rise to E-series and D-series resolvins, respectively, that both appear to have anti-inflammatory effects (Calder, 2006).

Studies on mice suggest that the FA composition of the maternal diet has perinatal programming effects on the offspring's immune response, although different studies have yielded contradictory results (Lauritzen et al., 2011; van Vlies et al., 2011). Knowledge of the effect of FA composition of sows' perinatal diet on piglet immunity post-weaning is limited, and the present study aimed to investigate whether the immunocompetence of piglets at weaning was modulated by including different sources of n-3 PUFA (fish oil, linseed oil or echium oil) in the gestation and lactation feed of the sow. These oils have a different composition of the n-3 PUFA fraction, and therefore each may influence eicosanoid production in a specific manner.

Fish oil is rich in EPA and DHA, which both exert immunomodulating effects, while linseed oil is rich in α-linolenic acid

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(C18:3n-3, ALA), which may be converted to EPA and DHA, but may also inhibit the production of ARA from linoleic acid (C18:2n-6, LA) by competing for the Δ6-desaturase enzyme. Echium oil contains not only ALA, but also stearidonic acid (C18:4n-3, SDA) which can be converted to EPA and DHA, and γ-linolenic acid (C18:3n-6, GLA) which can be elongated to the eicosanoid precursor DGLA. We hypothesized that supplementing these PUFA during the period of development will change the FA composition of the immune cells and alter their function later in life.

Materials and methods

The trial was conducted at the Institute for Agricultural and Fisheries Research (ILVO) in Melle, Belgium. The institutional and national guidelines for the care and use of animals were followed. All experimental procedures involving animals were approved by the Ethical Committee of ILVO (approval number EC 2010/131).

Animals and diets

Thirty-two pregnant sows (Rattlerow-Seghers hybrids, inseminated with Piétrain pig semen; parity 1–12) entered the experiment in two groups of 16 sows, and within each group the sows were allocated to one of four experimental diets (four sows per diet), so that for the different diets, the sows were balanced for parity and bodyweight. The experimental diets comprised a palm oil-based diet (control), a 1% linseed oil, 1% echium oil (Incromega V3, Croda Europe) and a 1% fish oil (INVE België) diet (Tables 1 and 2). All diets were formulated to contain a similar amount of LA (14 g/kg) and were supplemented with vitamin E (75 mg/kg).

The sows were fed the diets from day 73 of gestation (i.e. the third trimester) and during lactation, until weaning at 4 weeks. This corresponds with the main period of development of the pig immune system, as at birth most components of the immune system of the piglet are present but functionally undeveloped, and several weeks of life are needed before the immune system becomes fully developed (Rooke and Bland, 2002).

Gestating sows were penned individually and fed 2.6 kg/day of the gestation diet. One week before the expected farrowing date, sows were transferred to the farrowing crates, and fed 3 kg/day of the lactation diet until farrowing. After farrowing, they received 0.25 kg extra feed per piglet born. Piglets were not offered creep feed during lactation. Both gestation and lactation feeds were prepared in two batches. Due to a manufacturing fault, the group 2 sows from the linseed oil diet were fed a gestation diet containing 2% linseed oil instead of 1%. The data from these sows were not omitted from the dataset, as no differences were observed on the FA composition of the blood of these sows at parturition and of their piglets at birth (Tanghe et al., 2013).

At weaning, four piglets per litter (two males, two females) were selected, with bodyweights close to the average piglet weight of the litter. The total number of selected piglets was 127 (57 males, 70 females), as one echium oil fed sow of group 1 had only three female piglets and no male piglets at weaning, and three linseed oil fed group 1 sows and one palm oil fed sow from group 2 had, respectively, only one or no male piglets, resulting in three or four selected female piglets, respectively. Piglets were housed with six littermates (four selected and two non-selected) per pen. All piglets were fed the same diet (Tables 1 and 2) from weaning until day 35 post-weaning, with soy oil as the main fat source. The diet was not supplemented with any n-3 PUFA oil.

Table 1
Ingredients of the sow gestation diet (fed from day 73 of gestation until 7 days before expected farrowing), sow lactation diet (fed from 7 days before farrowing and during lactation), and piglet weaner diet (fed from weaning until 35 days post-weaning).

Ingredients, g/kg diet	Gestation diet	Lactation diet	Weaner diet
Wheat	250	250	150
Barley	250	150	274.3
Wheat middlings	120	50	–
Beet pulp	112	58.5	–
Corn	68.1	166	198.1
Soybean meal (45% crude protein)	48	134	150
Soy beans	32.7	34.5	51.8
Beet molasses	30	30	30
Alfalfa meal	18.3	63.7	–
Limestone	7.2	10.2	3.2
Monocalcium phosphate	–	–	5.3
Dicalcium phosphate	4.5	10.8	–
Benzoic acid[a]	5	–	–
Salt	5	4.3	2.5
Phytase (Natuphos, 5000 FTU/g)	0.1	0.1	0.1
L-Lysine HCl	1.5	2.1	4.4
L-Threonine	0.6	0.7	2.0
DL-Methionine	0.2	0.4	2.1
L-Valine	–	0.7	1.1
L-Tryptophan	–	0.1	0.8
Vitamin and mineral mix[b]	10	10	60
Starpro 40[c]	–	–	40
Nutrisure[d]	–	–	10
Oil inclusion[e]	36.8	24.2	14.3

[a] VevoVitall (DSM Nutritional Products).
[b] For the gestation and lactation diet of the sows, the vitamin and mineral premix provided the following quantities of vitamins and minerals per kilogram of diet: vitamin A, 3600 μg; vitamin D3, 50 μg; vitamin E, 75 mg; vitamin K, 1.01 mg; vitamin B1, 1.50 mg; vitamin B2, 5 mg; vitamin B5, 18 mg; vitamin B6, 4 mg; vitamin B12, 0.03 mg; vitamin PP, 25 mg; choline, 433 mg; folic acid, 3 mg; biotin, 0.3 mg; ethoxyquin, 0.55 mg; BHT, 0.50 mg; Ca, 888.5 mg; Mg, 164.4 mg; Fe, 150 mg; Cu, 15 mg; Mn, 50 mg; Zn, 100 mg; I, 2.0 g; Se, 0.4 mg. For the weaner diet of the piglets, the premix contained 80% dairy products and 20% vitamin and mineral premix, providing the following quantities of vitamins and minerals per kilogram of diet: vitamin A, 4500 μg; vitamin D3, 50 μg; vitamin E, 100 mg; vitamin K, 2 mg; vitamin B1, 2.5 mg; vitamin B2, 7.5 mg; vitamin B5, 20 mg; vitamin B6, 5 mg; vitamin B12, 0.04 mg; vitamin C, 100 mg; vitamin PP, 30 mg; choline, 324 mg; folic acid, 3 mg; biotin, 0.15 mg; Ca, 516 mg; P, 419 mg; Mg, 165 mg; Na, 353 mg; Cl, 1375 mg; K, 1227 mg; S, 234 mg; Fe, 100 mg; Cu, 160 mg; Mn, 60 mg; Zn, 100 mg; I, 2 mg; Se, 0.4 mg.
[c] Protein concentrate (DSM Nutritional Products).
[d] A mixture of calcium salts of the following organic acids: lactic acid, formic acid, citric acid monohydrate, orthophosphoric acid, propionic acid (DSM Nutritional Products).
[e] Details on oil inclusion are presented in Table 2.

Experimental and analytical procedures

To determine the effect of FA composition of the maternal diet on the antigen-specific immune response, the four selected piglets per litter were immunized with bovine thyroglobulin immediately after weaning (day 0). The piglets were injected IM with 1 mL of an emulsion of equal volumes of phosphate buffered saline (PBS) containing 1 mg bovine thyroglobulin (T-1001; Sigma-Aldrich) and incomplete Freund's adjuvant. A second identical injection was given at day 21 post-weaning. Blood samples were taken from each piglet by jugular venipuncture (Venosafe tubes with K_2EDTA, Terumo Europe) on days 0 (before immunization) and 7, 14, 21, 25, 28 and 35 post-weaning. All blood samples were centrifuged at 1800 *g* for 15 min; serum was then separated and stored at –20 °C until analysis.

Thyroglobulin-specific antibodies were measured by ELISA. The wells of a 96-well microtitre plate (NUNC, Polysorp Immuno Plates) were coated with thyroglobulin at a concentration of 10 μg/mL in coating buffer (carbonate-bicarbonate, 50 mM, pH 9.4) for 2 h at 37 °C. The remaining binding sites were blocked overnight with 0.2% Tween 80 in PBS at 4 °C. Subsequently, the sera were added for 1 h at 37 °C in a series of 2-fold dilutions in ELISA dilution buffer (PBS, pH 7.4 + 0.2% Tween 20 + 3% BSA), starting at a dilution of 1/10.

The plates were next incubated for 1 h with an optimal dilution of anti-pig immunoglobulins (Ig) IgG, IgA or IgM specific Mab (Van Zaane and Hulst, 1987), biotinylated rabbit anti-mouse Ig (Zymed Laboratories) and peroxidase conjugated streptavidin (DAKO, Prosan). Between each step, the plates were washed with washing buffer (PBS + 0.2% (v/v) Tween 20). Finally, ABTS-solution containing H_2O_2 (Roche Diagnostics) was added and after 30 min incubation the optical density (OD) was measured at 405 nm (OD_{405}). Cut-off values were calculated as the mean OD_{405} of all sera (dilution 1/10) on day 0 increased with three times the standard deviation. The antibody titre was the inverse of the highest dilution which still had an OD_{405} higher than the calculated cut-off values.

To determine the effect of the FA composition of the maternal diet on the level of the acute phase protein (APP) serum amyloid A (SAA), extra blood samples from one randomly selected piglet per litter were taken 1 day after each immunization with bovine thyroglobulin, resulting in four blood samples per animal for SAA analysis (days 0, 1, 21 and 22 post-weaning). The serum concentrations of SAA were measured using a commercially available ELISA kit (Phase Range SAA ELISA kit; Tridelta Development). The results were expressed as μg SAA per mL serum. Four piglets (two piglets from echium oil fed group 1 sows and two piglets from linseed oil fed sows, one from each group) had high SAA concentrations on day 0 (2–8 fold higher than the mean SAA concentration at day 0). These high concentrations probably resulted from a stressor outside the experiment, and as these data could have concealed a possible dietary effect on day 1, the data from these piglets were omitted.

Piglets were weighed at weaning and 14 and 35 days later. Total weight gain and average daily gain was calculated per piglet for the different time spans. Feed intake was registered per pen, and the average daily feed intake per piglet (feed intake per pen divided by number of days × number of piglets per pen) and the feed conversion ratio (feed intake per pen divided by total weight gain per pen) were calculated.