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Humoral defence improvement and haematopoiesis stimulation in sows and offspring by oral supply of shark-liver oil to mothers during gestation and lactation

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Shark-liver oil (SLO) contains two bioactive lipids: alkylglycerols and *n*-3 PUFA. Alkylglycerols have immunostimulating and haematopoietic properties, while *n*-3 PUFA are essential for optimal neonatal development. We investigated the beneficial effects of dietary supplementation with 32 g SLO/d to twelve pregnant and then lactating sows (from day 80 of pregnancy to weaning) on the growth and immune status of their offspring, compared with a control group. Sows were vaccinated against Aujeszky's disease 21 d before term. Blood samples were collected from sows before treatment, on delivery and 14 d later, and from five piglets per litter on days 2, 21 and 36 after birth; colostrum and milk samples were collected 12 h, 14 and 28 d postpartum. Compared with controls, supplemented sows had higher levels of both erythrocytes and Hb in their blood, and higher concentrations of IgG, alkylglycerols and *n*-3 PUFA in their mammary secretions. In piglets from supplemented sows, leucocytes and IgG were higher. Supplementation with SLO resulted in an increase in Aujeszky antibodies in both blood and colostrum of sows after vaccination, together with an increase in Aujeszky antibodies in piglet blood. Our findings demonstrate that improvement of both passive and active immune status in piglets is related to the consumption of alkylglycerols associated with *n*-3 PUFA in the sow diet. The overall improvement in offspring health status by SLO supplementation to the mother could be of interest for optimisation of the lipid diet during and after pregnancy.

Lipid diet: Maternal immunity transfer: Shark-liver oil: Alkylglycerols: *n*-3 PUFA

Shark-liver oil (SLO) is used in traditional medicine in Scandinavian countries for multifunctional properties such as stimulation of haematopoiesis and wound healing (Pugliese *et al.* 1998). SLO has been found to contain a group of ether-linked glycerols known as 1-*O*-alkylglycerols (alkyl-Gro). These natural ether lipids have multiple biological activities: they inhibit tumour growth (Brohult *et al.* 1978; Pedrono *et al.* 2004), reduce thrombocytopenia following radiation therapy for carcinoma of the uterine cervix (Brohult *et al.* 1977) and modulate endothelial permeability *in vitro* (Marigny *et al.* 2002). They also have beneficial effects *in vitro* on sperm motility and fertility (Cheminade *et al.* 2002). Furthermore, oral SLO also improves sperm function in boars (Mitre *et al.* 2004). In the field of immunity, alkyl-Gro enhance both macrophage activation (Yamamoto *et al.* 1988) and antibody production in rodents (Ngwenya & Foster, 1991; Chorostowska-Wynimko *et al.* 2001) and man (Brohult *et al.* 1972). Furthermore, oral treatment by synthetic alkyl-Gro in lactating rats greatly increases total IgM and IgG in plasma of pups (Oh & Jadhav, 1994). In addition to abundance in alkyl-Gro, SLO, like other fish oils, is also rich in long-chain *n*-3 PUFA. These essential fatty acids play a major role in development, notably of the nervous system (Voigt *et al.* 2002; Helland *et al.* 2003). Diet composition in fatty acids before and during lactation has a direct influence on the lipid composition of milk in various mammal species (Taugbol *et al.* 1993; Francois *et al.* 2003; Kitessa *et al.* 2004) and milk enrichment in long-chain *n*-3 PUFA improves neonate development (Rooke *et al.* 2001; Helland *et al.* 2003). Because of its dual content of alkyl-Gro and *n*-3 PUFA, SLO is of particular interest in nutrition.

The aim of the present study was to determine the effect of dietary supplementation with SLO in pregnant and lactating sows, with respect to offspring development and immune and haematological status of both mother and offspring. We showed that SLO supplementation of the mother's diet modified the lipid

Abbreviations: alkyl-Gro, 1-*O*-alkylglycerols; SLO, shark-liver oil.

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composition of colostrum and milk, together with their content of Ig, and improved both passive and active immunity in piglets. This supplementation with SLO also improved offspring growth.

Experimental methods

Materials

Shark-liver oil was obtained from Id-Mer (Lorient, France) after the removal of squalene from the raw oil of the Siki shark (*Centrophorus squamosus*). It contained undetectable amounts of vitamins A and E, and <0·13 μg vitamin D/100 g. This oil, from a single batch, contained 25 % (of the total weight) of alkyl-Gro as measured after saponification and separation from fatty acids. The alkyl chains, linked by an ether bond to the sn-1 position of the glycerol, were identified and quantified as described below and were composed of: C14 : 0 (2·7 %), C16 : 0 (13·7 %), C16 : 1*n*-7 (11·2 %), C18 : 0 (2·8 %) C18 : 1*n*-7 (8·3 %) and C18 : 1*n*-9 (58·8 %). Each other minor species individually represented less than 1 % of the total amount. Oil fatty acid composition, analysed after saponification and transmethylation as described below, is presented in Table 1.

1-*O*-Alkylglycerol 17 : 0 was kindly provided by Professor Françoise Heymans (Laboratoire de Pharmacochimie Moléculaire de l'Université de Paris VI, Paris, France). All solvents were obtained from Prolabo (Fontenay-sous-Bois, France). For TLC we used Whatman silica gel 60 Å LK6 plates, which were obtained from Interchim SA (Montluçon, France). For GC, the following equipment was used: GC 8000TOP from ThermoFinnigan France (Les Ulis, France); Chrompack CP-SIL-5CB capillary column (length 25 m, inside diameter 250 μm, film thickness 0·12 μm) for alkyl-Gro analysis was purchased from Varian France (Les Ulis, France) and BPX 70 capillary column (length 30 m, inside diameter 250 μm, film thickness 0·25 μm) for fatty acid content analysis was obtained from SGE (Villeneuve Saint Georges, France). Blood cell counts were performed by an ADVIA 120 haematology system validated for pig erythrocyte and leucocyte cell counts (Bayer Diagnostics SA, Puteaux, France).

Feeding and diet composition

Two basal diets were used for the present experiment: a gestation diet and a lactation diet, for which fatty acid contents are presented in Table 1. They consisted of (g/kg): wheat (221), corn (100), barley (337), wheat bran (150), soyabean cattle cake (90), corn oil (20), beetroot pulp (50) and minerals (32) for the gestation diet (digestible energy 12·93 MJ/kg; fat 3·9 %); and wheat (227), corn (120), barley (256), wheat bran (100), soyabean cattle cake (210), corn oil (20), treacle (30) and minerals (37) for the lactation diet (digestible energy 13·36 MJ/kg; fat 3·9 %). Gestation and lactation diets contained vitamin A (3 mg/kg), vitamin D (37.5 μg/kg) and vitamin E (43 mg/kg); these quantities were not altered by SLO supplementation. Sows received specific amounts of food in the following quantities: during gestation and until parturition, 2·7 kg/d (one meal); at lactation day

Table 1. Fatty acid and alkylglycerol daily supply provided by the diets

		Lactation			
	Gestation	Day 1	Day 2 to day 6	Day 7 to day 28	SLO
Fatty acids (g/d)					
14 : 0	–	–	–	–	0·3
16 : 0	13·7	18·1	23·3	28·5	3·8
16 : 1*n*-7	–	–	–	–	1·1
18 : 0	2·4	3·1	3·9	4·8	0·5
18 : 1*n*-9	21·6	29·3	37·7	46·0	7·8
18 : 1*n*-7	1·3	1·7	2·2	2·7	1·3
18 : 2*n*-6	48·7	61·9	79·6	97·3	0·2
18 : 3*n*-3	4·5	5·8	7·4	9·1	0·1
18 : 4*n*-3	–	–	–	–	2·6
20 : 1*n*-9	–	–	–	–	0·3
20 : 4*n*-3	–	–	–	–	2·4
20 : 5*n*-3	–	–	–	–	1·1
22 : 5*n*-3	–	–	–	–	1·0
22 : 6*n*-3	–	–	–	–	0·2
Others	2·4	2·7	3·5	4·2	0·3
Total	94·6	122·6	157·6	192·6	22·9
Σ*n*-3	4·5	5·8	7·4	9·1	7·4
Σ*n*-6	48·7	61·9	79·6	97·3	0·2
n-6 : *n*-3 (−SLO)	10·8	10·7	10·7	10·7	–
n-6 : *n*-3 (+SLO)	4·1	4·7	5·4	5·9	–
Alkyl-Gro (g/d)					
14 : 0	–	–	–	–	0·2
16 : 0	–	–	–	–	1·1
16 : 1*n*-7	–	–	–	–	0·9
18 : 0	–	–	–	–	0·2
18 : 1*n*-9	–	–	–	–	4·7
18 : 1*n*-7	–	–	–	–	0·7
Others	–	–	–	–	0·2
Total	–	–	–	–	8·0

SLO, shark-liver oil; alkyl-Gro, 1-*O*-alkylglycerols.

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1, 3·5 kg/d (two meals); from lactation day 2 until day 6, 4·5 kg/d (two meals); and from lactation day 7 until weaning, 5·5 kg/d (two meals).

Animal care

All animal procedures described followed established guidelines for the care and handling of laboratory animals and complied with the directives from the French Ministry of Agriculture. The animals were raised and cared for at the experimental rearing facility of the Institut National de Recherche Agronomique located at Saint Gilles, France.

Twenty-four crossbred pregnant sows (Large White × Landrace genetic lines) were paired according to age, body weight (250·1 (SE 10·4) and 208·2 (SE 9·2) kg at delivery and weaning, respectively) and parity, then randomly assigned from pairs into two groups (eight primiparous and four multiparous by group). The first group served as control and received standard gestation and lactation diets whereas the second group received a dietary supplementation of SLO (32 g/d), which was poured directly onto the standard diets every morning, from 5 weeks before parturition until weaning. This occurred 28 d after delivery by withdrawing piglets from the sows. Supplement of SLO increased digestible energy by +3·4 % and +1·6 % of daily intake in gestating and lactating sows, respectively. The sows were kept in single crates until day 110 of pregnancy and then moved to the farrowing pens. Temperature for the newborn piglets was maintained at a constant 35°C. The climate in the sow accommodation and the farrowing unit was maintained at a temperature of 19 ± 2°C and 60–80 % relative humidity by means of an air conditioning system. A 12 h light:12 h dark cycle was applied.

At delivery, and for each litter, all piglets were weighed and five were selected according to their birth weight: the one closest to the mean, the two closest to the mean ± 0·5 SD and the two closest to the mean ± 1 SD. All measurements were performed on these same five piglets per litter throughout the whole experiment. Except for the selected piglets, litter sizes were standardised by adoption procedures 24 h after delivery: finally, there were 10·5 (SE 0·22) and 10·0 (SE 0·26) offspring per litter for the control group and the supplemented group, respectively. All piglets were weighed once a week from parturition until weaning. Sows were vaccinated against Aujeszky's disease (Pseudorabies Virus) 21 d before parturition (Geskypur vaccine, Merial, Lyon, France). Piglets received an Fe-dextran injection and males were castrated at day 7 after birth.

Sample collection

Blood samples were taken from sows before supplementation, at parturition and lactation day 14, and from piglets on days 2, 21 and 36 after birth. Blood samples were taken by puncture in the jugular vein for both sows and piglets. Samples were collected in EDTA-coated vials for blood cell counts and in dry vials for serum separation and Ig assays. Serum was stored at −32°C until assay. For colostrum and milk sampling, piglets were removed from the sow 1 h before and lactation was induced by oxytocin injection. A sample of colostrum was collected 12 ± 1 h after birth of the first piglet, and a sample of mother's milk was taken on lactating days 14 and 28 after milking, from every teat. Raw colostrum or milk samples were processed as follows: 5 ml of raw material was centrifuged (2000 ***g*** at 4°C, 20 min); supernatant cream was collected and stored at −32°C before lipid extraction and analysis. Skimmed colostrum and milk were then ultracentrifuged (85 000 ***g*** at 4°C, 1 h). The resulting supernatant (lactoserum) was collected and stored at −32°C until assay.

Lipid extraction from colostrum and milk

Lipids from cream samples were extracted according to Bligh & Dyer (1959). Total lipids were weighed and dissolved in chloroform for further analysis.

Analysis of ether lipid moieties from colostrum and milk fat

Seventy milligrams of total-lipid extract from each sample was weighed and dried. Then 30 μg of 17 : 0 alkyl-Gro was added as an internal standard. This mixture was dissolved in 10 ml 0·5 M-methanolic KOH, incubated for 1 h at 70°C and then allowed to cool at room temperature. Unsaponifiable matter was extracted from the samples by using two lots of diethyl ether (20 ml each). Alkyl-Gro was separated from the unsaponifiable fraction on silica gel plates by TLC (hexane–diethyl ether–methanol–acetic acid, 80 : 20 : 10 : 1 v/v), eluted from silica with diethyl ether, and then dried. The extracts were acetylated according to the method described by Kumar *et al.* (1983) and slightly modified. Briefly, lipid extracts were incubated for 2 h at 120°C in 0·5 ml of an acetic anhydride–acetic acid mixture (3 : 2, v/v). Then, 3 ml chloroform was added and the resulting solution was washed three times with water and twice with 0·2 M-Na_2CO_3. The solution containing the resulting alkyldiacetylglycerols was dried and re-suspended in a small volume of hexane. The samples were analysed by GC (200 to 250°C; 2°C/min; He 1 ml/min; column described earlier). Acetylated alkyl-Gro species were identified according to their retention time by comparison with standards and were quantified using the alkyl-Gro 17 : 0 internal standard.

Analysis of fatty acids from colostrum and milk fat

Five milligrams of the total-lipid extract from each sample was dissolved in 1 ml 0·5 M-methanolic KOH with 400 μg of 17 : 0 fatty acid added as an internal standard. Samples were incubated for 45 min at 70°C, then the fatty acids were methylated by addition of 1 ml methanolic BF3 (15 min at 70°C). The samples were allowed to cool at room temperature and 3 ml H_2O was added. Fatty acid methyl esters were extracted with diethyl ether, dried under N_2 and dissolved in n-pentane for further analysis by GC (120 to 210°C; 4°C/min; He 1 ml/min; column described earlier). Fatty acids were identified according to their retention time compared with those of standards analysed in the same conditions and were quantified using the fatty acid 17 : 0 internal standard.

Immunoglobulin assays

Assays for IgG, IgA and IgM were performed using specific ELISA quantification kits purchased from Bethyl Laboratories, Inc. (Montgomery, TX, USA) and following kit protocols. Serum and lactoserum dilutions were as follows: serum samples were diluted to 1 : 250 000 and 1 : 20 000 for IgG and IgM,

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respectively; lactoserum samples from colostrum were diluted to 1 : 250 000 and 1 : 10 000 for IgG and IgA, respectively; and lactoserum samples from milk were diluted to 1 : 10 000 for both IgG and IgA.

Quantification of anti-Aujeszky antibodies was performed using kits obtained from IDEXX SARL (Cergy Pontoise, France) and by following kit instructions except for a slight modification of the sample dilutions, which were adapted to kit sensitivity: samples were diluted to 1 : 4000 and 1 : 50 000 for serum samples and lactoserum samples from colostrum, respectively. Anti-Aujeszky antibody concentrations (arbitrary units) were established by comparison of sample levels with levels of negative (Neg) and positive (Pos) standards according to the following formula: concentration $= (OD_{\text{sample}} - OD_{\text{Neg}})/(OD_{\text{Pos}} - OD_{\text{Neg}})$, with OD being the optical density read at 620 nm.

Statistical analysis

Data are presented as means with their standard errors of the indicated number of animals or litters, each measurement being performed in triplicate. Statistical analysis of the data was performed with GraphPad Prism 4·01 (GraphPad Software, San Diego, CA, USA) or StatPak 4·1 (NorthWest Analytical Inc., Portland, OR, USA). The significance of the differences observed between groups for single time measurements was assessed by the two-sided unpaired Student t test or the Mann–Whitney non-parametric test according to n value ($n < 20$). For measurements repeated over time, two-way or three-way repeated-measures ANOVA was used for sows (factors: supplementation and time) or for piglets (factors: supplementation, time and litter), respectively. When first time points presented differences, they were included as covariates by linear regressions. The effect of time was assessed by linear regressions which were further compared in supplemented and control groups. The type of test used is indicated in the text whenever it was significant, using a two-sided test or otherwise indicated. For the zootechnical offspring results, all animals were included in the data except adopted piglets.

Results

Effects of shark-liver oil on erythrocytes and immunoglobulin in sows

Dietary supplementation with SLO enhanced erythrocyte number and, to a lesser extent, Hb concentration in sow blood (Fig. 1). We observed a significant difference in erythrocytes and Hb (comparison of regressions by one-sided test: $P=0{\cdot}015$ and $P=0{\cdot}035$, respectively) between groups over time; mean values in the supplemented group were 10·1 and 8·6 % greater for erythrocytes (ANOVA: $P=0{\cdot}027$) and 8 and 6 % greater for Hb (ANOVA: $P=0{\cdot}037$) than those of the control group at parturition and 2 weeks later, respectively. On the other hand, leucocytes were not influenced by diet at any time in sows (data not shown).

We also studied the effects of the supplementation on Ig levels in blood. SLO supplementation resulted in a significant increase over time (ANOVA: treatment, $P=0{\cdot}049$; time, $P<0{\cdot}001$) in specific Ig against antigens of the pathological agent responsible for Aujeszky's disease after standard vaccination (Fig. 2(A)). However, no variations in the concentrations of total IgG and IgM in sow serum were observed (data not shown).

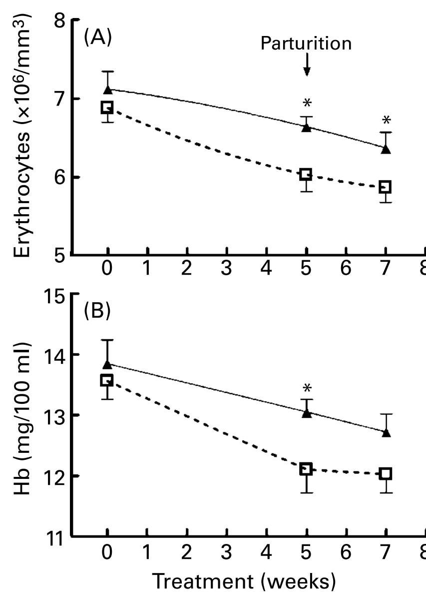

Fig. 1. Effect of dietary supplementation in sows fed a basal diet (CTR; □) or supplemented with shark-liver oil (SLO; ▲) on erythrocyte concentration (A) and Hb concentration (B). Measurements were made at day 0 of treatment, at parturition and 2 weeks after parturition. Values are means with their standard errors shown by vertical bars (n 12). Overall differences were assessed by comparing regressions using all data, including the first time point ($P=0{\cdot}015$ and 0·035 for erythrocytes and Hb, respectively; one-sided test). Mean values were significantly different SLO *v.* CTR (Mann–Whitney test): *$P<0{\cdot}05$.

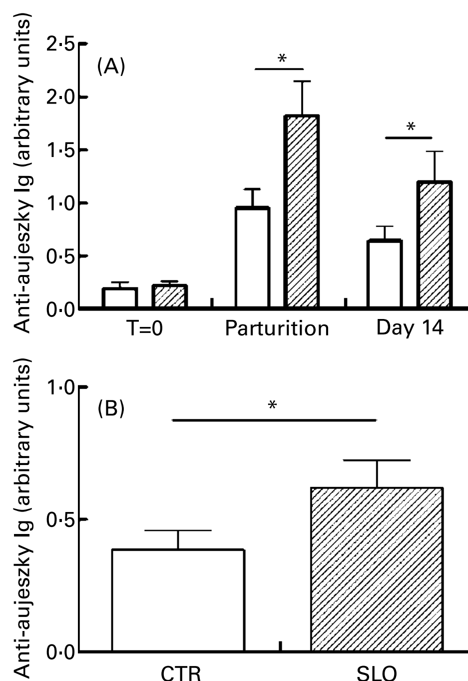

Fig. 2. Effect of dietary supplementation in sows fed a basal diet (CTR; □) or supplemented with shark-liver oil (SLO; ▨) on concentration of anti-Aujeszky antibodies in sow serum (A) and sow colostrum (B). Values are means with their standard errors shown by vertical bars (n 12). Mean values were significantly different SLO *v.* CTR (Mann–Whitney): *$P<0{\cdot}05$. Significance of the difference over time for anti-Aujeszky antibodies in serum (repeated-measures ANOVA): $P<0{\cdot}05$.

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Influence of shark-liver oil supplementation on colostrum and milk

Ig. SLO supplementation had a significant positive influence on IgG concentration in lactoserum: IgG levels in supplemented sows were 112 % (Mann–Whitney: $P < 0.0001$) greater in colostrum, and 58 % and 83 % (ANOVA: treatment, $P = 0.021$) greater in milk at day 14 and day 28, respectively, compared with controls (Fig. 3(A and C)). On the other hand, the levels of IgA in colostrum and milk were not modified by the dietary supplementation (Fig. 3(B and D)). Moreover, supplementation with SLO also influenced the specific immunity in colostrum since the concentration of anti-Aujeszky antibodies in colostrum of the supplemented group was significantly greater (+61 %) than in the control group (Mann–Whitney: $P = 0.036$; Fig. 2(B)).

Lipid composition. At day 28 after parturition, we observed that SLO supplementation enhanced the overall lipid concentration in milk by 26 % compared with control animals (Mann–Whitney: $P = 0.023$; Table 2). We also studied the effects of SLO supplementation on the lipid composition of colostrum and milk with respect to fatty acids and alkyl-Gro, the two major constituents of SLO (Table 2). The prominent and constant influence of supplementation was the appearance of C16 : 1 alkyl chains in colostrum and milk, contrasting with its absence in controls. We also observed a 27 % (Mann–Whitney: $P = 0.038$) increase in total alkyl-Gro in the milk of the supplemented group at day 28, resulting from an overall increase of individual alkyl-Gro species which reached 30 % for C18 : 1. In contrast, the increase in the overall concentration of fatty acids in colostrum and milk did not reach significance in the supplemented group at any time studied. However, the distribution of fatty acids among classes revealed higher ratio of *n*-3 PUFA to overall measured fatty acids in mammary secretions of supplemented sows at any time compared with controls (linear regression comparison: $P < 0.05$; Table 2), although the differences did not increase, but rather decreased over time. No changes were observed for other fatty acid classes except a decrease in the ratio of *n*-6 PUFA to overall measured fatty acids at day 28 (Mann–Whitney one-sided test: $P = 0.034$).

Modulation of the immune system of newborn piglets by diet supplementation of the mother with shark-liver oil

We studied the impact of the mother's diet on both the active and the passive immune system of the piglets. Overall leucocyte levels, as well as main classes of leucocyte, increased in the blood of piglets from supplemented sows (Fig. 4). The strongest effect was observed on monocytes, which showed an increase of 44 % at day 36 (Fig. 4(D)).

Ig concentrations in piglet blood were differentially influenced by the mother's diet (Fig. 5). We observed an overall significant increase in IgG concentration in offspring sera from the SLO-supplemented group compared with the control animals over time (ANOVA: treatment, $P < 0.001$; time, $P < 0.001$; Fig. 5(A)). IgM concentrations were not significantly affected by the mother's diet (Fig. 5(B)). Sows are usually vaccinated against Aujeszky's disease 21 d before parturition, and this results in the appearance of anti-Aujeszky-specific antibodies in offspring after colostrum intake. Interestingly, there was more anti-Aujeszky antibody in piglets from the supplemented group (ANOVA: treatment, $P = 0.011$; time, $P < 0.001$), indicating that this passive immunity was improved by SLO: total anti-Aujeszky antibodies in piglet serum of the supplemented group was 61 % (Student *t* test: $P = 0.0087$) and 19 % (Student *t* test: $P = 0.038$) greater than control at day 2 and day 36 after parturition, respectively (Fig. 6).

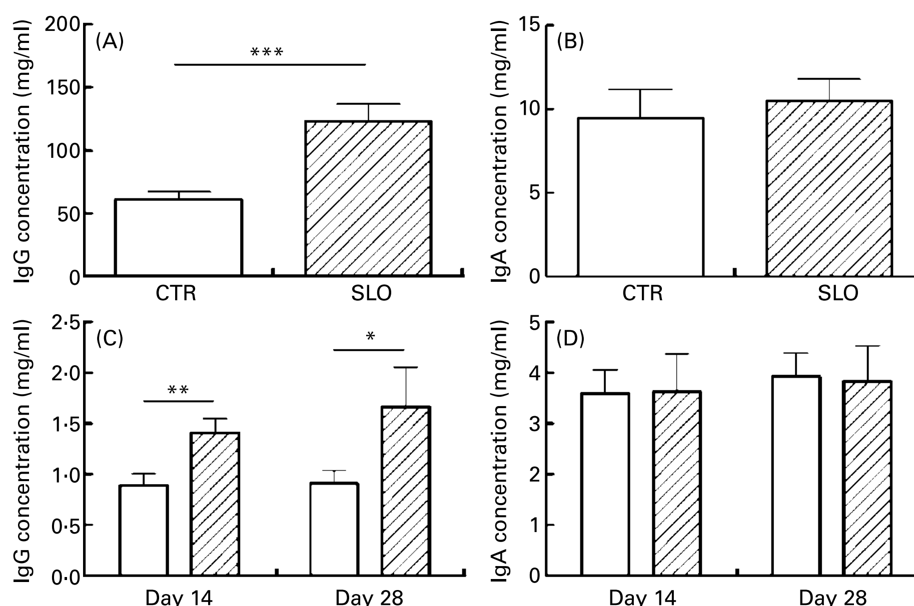

Fig. 3. Effect of dietary supplementation in sows fed a basal diet (CTR; □) or supplemented with shark-liver oil (SLO; ▨) on IgG and IgA concentrations in lactoserum from colostrum (A and B) and milk (C and D). Measurements were made 12 h after parturition for colostrum, and at days 14 and 28 after parturition for milk in CTR and SLO groups. Values are means with their standard errors shown by vertical bars (*n* 12). Mean values were significantly different SLO *v.* CTR (Mann–Whitney test): *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Significance of the difference over time for IgG in milk (repeated-measures ANOVA): $P < 0.05$.

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Table 2. Effect of dietary supplementation with shark-liver oil on alkylglycerol and fatty acid concentrations, and total lipid amounts, in sow colostrum and milk
(Mean values with their standard errors)

	Colostrum				Milk at day 14				Milk at day 28			
	CTR		SLO		CTR		SLO		CTR		SLO	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Alkyl-Gro (μg/ml)												
14:0	19·17	3·20	12·16	1·78	22·63	2·22	25·60	2·26	16·8	1·60	22·80	3·77
16:0	53·72	6·56	31·58*	5·49	103·99	8·79	109·45	9·94	62·33	3·55	73·82	7·91
16:1	ND	ND	5·06***	0·81	ND	ND	4·13***	0·30	ND	ND	3·96***	0·21
18:0	30·00	2·73	17·88*	3·63	32·30	3·39	29·60	4·39	16·34	0·49	19·27	1·77
18:1	84·45	7·41	68·40	12·30	97·80	8·45	106·90	13·76	50·37	2·21	65·36*	5·07
Total	187·33	17·94	135·09	23·8	256·71	20·16	275·69	27·87	145·22	5·75	185·20*	17·22
Fatty acids (mg/ml)†												
14:0	0·67	0·10	0·52	0·09	1·56	0·10	1·91*	0·11	1·25	0·08	1·50	0·13
16:0	10·72	1·21	7·48	1·44	17·60	1·04	19·54	1·75	12·82	0·71	14·21	0·87
16:1*n*-9	0·53	0·05	0·42	0·08	0·38	0·06	0·46	0·08	0·18	0·02	0·20	0·03
16:1*n*-7	1·21	0·18	1·04	0·27	4·24	0·33	4·90	0·25	3·14	0·24	3·51	0·22
18:0	3·04	0·37	2·01	0·44	4·19	0·68	4·38	0·82	2·15	0·14	2·30	0·18
18:1*n*-9	15·22	1·66	11·40	2·77	25·69	3·35	30·14	5·04	14·24	1·08	16·52	1·24
18:1*n*-7	1·27	0·14	0·96	0·19	1·79	0·22	2·14	0·35	0·99	0·07	1·16	0·06
18:2*n*-6	12·89	1·36	9·04	1·37	11·99	1·32	13·75	2·28	7·54	0·48	7·98	0·48
18:3*n*-3	1·18	0·14	0·86	0·13	1·06	0·11	1·26	0·21	0·67	0·05	0·73	0·05
20:1*n*-9	0·15	0·02	0·17	0·04	0·30	0·05	0·40	0·07	0·17	0·01	0·25	0·01
20:2*n*-6	0·27	0·03	0·17*	0·03	0·37	0·06	0·42	0·09	0·20	0·02	0·21	0·02
20:4*n*-6	0·46	0·05	0·32*	0·05	0·36	0·04	0·43	0·06	0·20	0·02	0·21	0·01
22:5*n*-3	0·21	0·03	0·15	0·02	0·17	0·02	0·20	0·04	0·08	0·01	0·09	0·01
22:6*n*-3	0·038	0·003	0·038	0·007	0·027	0·004	0·036	0·005	0·013	0·002	0·017	0·002
Total lipids	57·7	7·0	40·2	7·6	83·6	8·1	94·0	12·9	59·9	3·7	75·6*	3·9
%*n*-3	2·74	0·12	3·10	0·14	1·65	0·07	1·95	0·16	1·65	0·07	1·85	0·15

CTR, control group; SLO, shark-liver oil supplemented group; alkyl-Gro, 1-*O*-alkylglycerols; ND, not detectable.
Mean values were significantly different SLO *v.* CTR (two-sided Mann–Whitney test, *n* 12): $^{*}P<0·05$, $^{***}P<0·001$.
† Only selected fatty acids are represented (>0·5 % of total fatty acids).

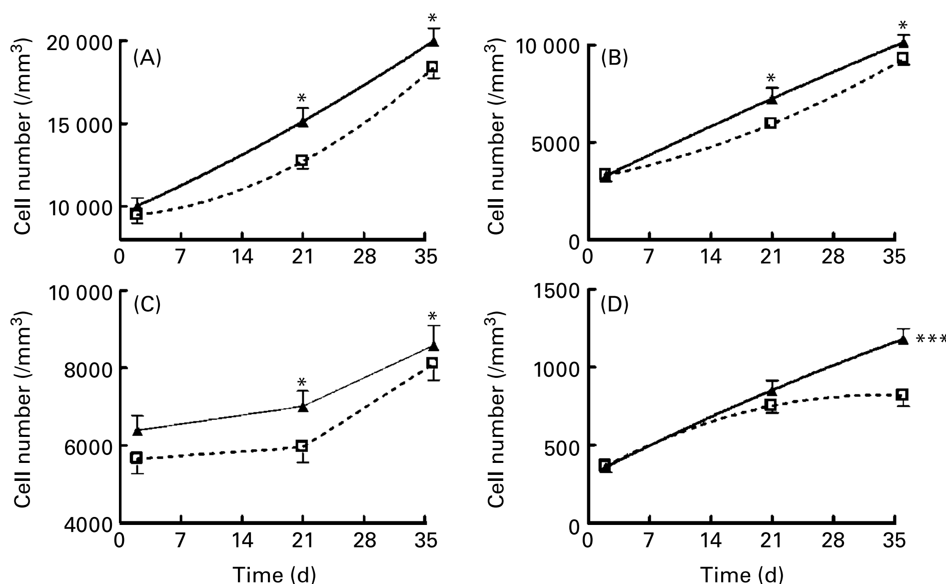

Fig. 4. Leucocyte counts in piglets from sows fed a basal diet (CTR; □) or supplemented with shark-liver oil (SLO; ▲). Counts are shown for leucocytes (A), lymphocytes (B), neutrophils (C) and monocytes (D). Measurements were made from blood samples collected at days 2, 21 and 36 after birth. Values are means for each group with their standard errors shown by vertical bars (*n* 60). Overall differences were assessed by comparing regressions using all data, including the first time point ($P=0.0115$, $P=0.0189$, $P=0.049$ and $P<0.001$ for (A), (B), (C) and (D) respectively). Mean values were significantly different SLO *v.* CTR (student's *t* test): $^{*}P<0·05$, $^{***}P<0·001$.

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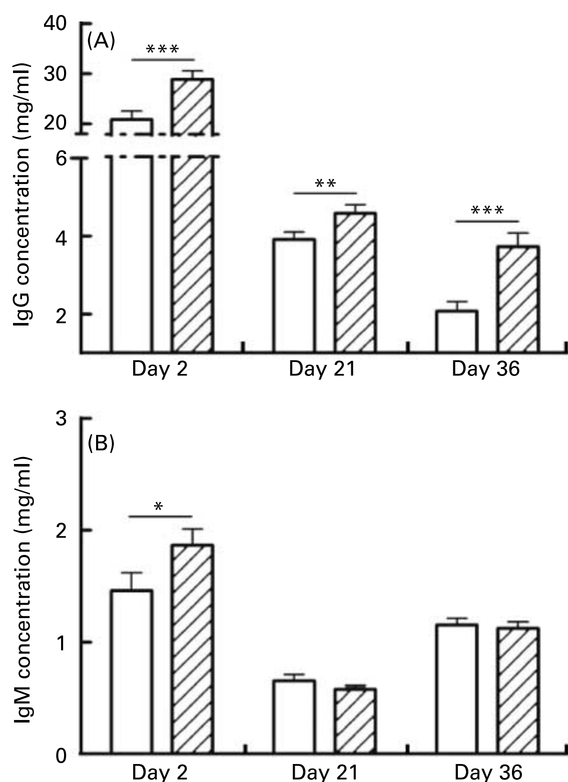

Fig. 5. Concentration of IgG (A) and IgM (B) in piglet serum from sows fed a basal diet (CTR; □) or supplemented with shark-liver oil (SLO; ▨). Measurements were made from blood samples collected at days 2, 21 and 36 after birth. Values are means for each group with their standard errors shown by vertical bars (*n* 60). Mean values were significantly different SLO *v.* CTR (Student *t* test): $^*P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$. Significance of the differences over time for IgG concentration (repeated measures-ANOVA): $P<0{\cdot}001$.

Improvement of weight and growth in piglets from shark-liver oil-supplemented sows

Piglet growth was significantly higher (regression comparisons: $P=0{\cdot}0011$) in the supplemented group and did not slow down between day 21 and day 28, in contrast with control piglets (Fig. 7). The mean litter size in the treated group was smaller (Table 3). This difference, although without statistical significance, could have influenced piglet birth weights, which were higher (+11 %; Student *t* test: $P=0{\cdot}036$) in litters from supplemented sows, since birth weights were negatively correlated to litter size ($P<0{\cdot}001$). At the end of observation piglets from supplemented sows had a 5 % higher weaning weight as well (Student *t* test: $P=0{\cdot}0404$; Table 3), which was also correlated to birth weight ($P<0{\cdot}001$).

Discussion

SLO is a natural source of both alkyl-Gro and *n*-3 PUFA. Alkyl-Gro have biological activities resulting in the improvement of different immune responses, and *n*-3 PUFA are determinant for healthy postnatal development. In the present study we demonstrate that dietary supplementation of gestating and lactating sows with SLO increased erythrocytes in sows and leucocytes in piglets. This supplementation also resulted in enrichment of

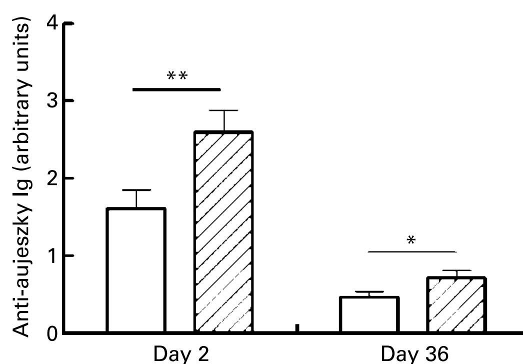

Fig. 6. Anti-Aujeszky antibody concentration in piglet serum from sows fed a basal diet (CTR; □) or supplemented with shark-liver oil (SLO; ▨). Measurements were made from blood samples collected on days 2 and 36 after parturition. Values are means for each group with their standard errors shown by vertical bars (*n* 60). Mean values were significantly different SLO *v.* CTR (Student *t* test): $^*P<0{\cdot}05$, $^{**}P<0{\cdot}01$.

mammary secretions in both alkyl-Gro and *n*-3 PUFA, improving both active and passive immune defences.

Alkyl-Gro from SLO have a wide spectrum of effects (Pugliese *et al.* 1998). Their improvement of the immune responses is well documented: they potentiate macrophage activation *in vitro* (Yamamoto *et al.* 1988) and increase antibody production by oral administration alone (Brohult *et al.* 1972) or in synergy with lactic acid bacteria (Chorostowska-Wynimko *et al.* 2001). The importance of *n*-3 PUFA on early development is also well established: when administrated to lactating mothers they have positive effects on offspring neurodevelopment (Voigt *et al.* 2002; Helland *et al.* 2003), sight (Hoffman *et al.* 2000), general movements (Bouwstra *et al.* 2003) and growth (Rooke *et al.* 2001). Dietary *n*-3 PUFA appear to influence the immune system mainly by inhibiting effects in various species (Blok *et al.* 1996; Calder, 1998; Anderson & Fritsche, 2002); in pigs they suppress the proliferation of T and B lymphocytes (Liu *et al.* 2003).

In the control group of gestating sows we observed a drop in erythrocytes during pregnancy and lactation. This trend to gestation- and lactation-linked anaemia is frequently observed in man (Bashiri *et al.* 2003) and pigs. In supplemented sows

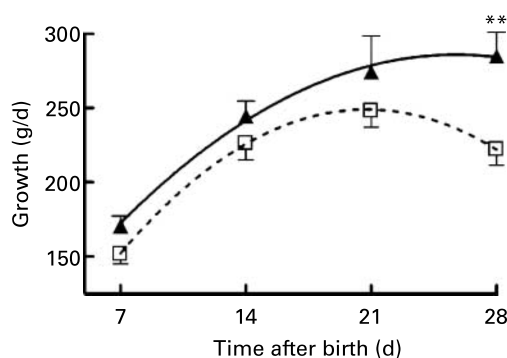

Fig. 7. Effect of sow diet on piglet growth. Sows were fed a basal diet (CTR; ▲) or supplemented with shark-liver oil (SLO; □). Values are means for each group with their standard errors shown by vertical bars (*n* 60). Overall difference was assessed by comparing regressions using all data, including first time point ($P=0{\cdot}0011$). At day 36 after birth, the differences over time were significantly different, SLO *v.* CTR (repeated measures-ANOVA followed by Student *t* test): $^{**}P<0{\cdot}01$.

Table 3. Effect of sow dietary supplementation with shark-liver oil on offspring weight at birth and weaning
(Mean values with their standard errors for the number of animals indicated)

		CTR			SLO		
		Mean	SE	*n*	Mean	SE	*n*
Before standardisation	Litter size	13·3	0·7	12	10·7	1·0	12
	Birth weight (kg)	1·33	0·04	134	1·48*	0·05	127
After standardisation	Litter size	10·5	0·22	12	10·0	0·26	12
	Weaning weight (kg)	7·35	0·12	101	7·74*	0·10	100

CTR, control group; SLO, shark-liver oil-supplemented group.
Mean values were significantly different SLO *v.* CTR (two-sided Student *t* test): $^*P<0·05$.

the decrease in erythrocytes was partly prevented by enhancement of their concentration and, to a lesser extent, of Hb concentration. These data are consistent with early findings by Linman *et al.* (1958) of the erythropoietic stimulating activity of C18 : 0 alkyl-Gro. SLO supplementation also had effects on sow immune responses: although it did not alter the level of overall Ig, it had a significant positive effect on specific Ig induced by vaccination against Aujeszky's disease. This confirms the improving effect of oral alkyl-Gro or SLO on humoral immunity (Chorostowska-Wynimko *et al.* 2001). Sow placenta is not permeable to Ig; therefore IgG transfer to offspring is highly dependent on the selective IgG transport mechanism from blood to secretions across secretory epithelial cells of the colostrum-forming mammary gland (Salmon, 1999). We observed higher levels of IgG in colostrum and milk due to supplementation. As total Ig in serum of supplemented sows were not increased and since their transfer in mammary secretions is governed by specific affinity mechanisms (Salmon, 1999), the overall IgG rise observed in colostrum and milk of supplemented sows suggests a higher level of transfer of IgG from blood plasma to colostrum and milk. As expected, Aujeszky's specific antibodies were also enhanced and reached a 61 % increase in the colostrum of sows receiving dietary SLO supplementation.

Because both alkyl-Gro and *n*-3 PUFA can bring about health benefits, it was of interest to determine whether these lipids could be significantly incorporated in mammary secretions. Alkyl-Gro are natural constituents of human and bovine milk with a preponderance of chimyl, bathyl and selachyl alcohols (Hallgren & Larsson, 1962). Therefore the transfer of alkyl-Gro from the diet to mammary secretions was assessed by the appearance of C16 : 1 alkyl chains in milk and colostrum after supplementation. This alkyl-Gro species is absent in lipids of control animal mammary secretions while it represents the second most abundant alkyl chain in SLO, and therefore may be considered a tracer of alkyl-Gro transfer. Moreover, total alkyl-Gro were increased in milk after 9 weeks of supplementation. Thus piglets from supplemented sows received higher amounts of alkyl-Gro via mother's milk; however, this transfer could have started earlier during pregnancy. The supplementation also influenced the *n*-3 PUFA composition of overall mammary secretions. Previous data showed that oral *n*-3 PUFA administration results in the increase of this fatty acid class in milk (Taugbol *et al.* 1993; Francois *et al.* 2003; Kitessa *et al.* 2004). In our study the *n*-3 PUFA class was increased in milk, resulting in lower *n*-6 : *n*-3 ratio in supplemented sow mammary secretions. This could also provide beneficial dietary effect on piglets. We also observed a trend to an overall lower level of lipids in the colostrum of supplemented sows; however, since this trend was mainly non-significant, we attributed it to random fluctuations.

Sow supplementation with SLO had a positive impact on the health and immune status of the neonates. From day 2 to day 36 after birth, leucocyte counts increased with respect to lymphocytes, neutrophils and prominently monocytes. Furthermore, neutrophil levels were higher right from the birth, suggesting an effect of the treatment starting *in utero*. Serum IgG concentrations were also higher in piglets from the supplemented group. The rise in overall and Aujeszky-specific Ig together with haematopoietic activity emphasised the dual effect of sow supplementation on both passive and active immunity in offspring. In a previous study, synthetic alkyl-Gro administrated *per os* to lactating rats were also incorporated in mother's milk and enhanced blood granulocyte concentration and Ig levels in blood of suckling pups (Oh & Jadhav, 1994). We show here for the first time that natural alkyl-Gro can improve the whole leucocyte population in blood as well as specific antibody concentration in offspring after immunisation of the gestating mothers.

In addition to these physiological modifications, in the present study we observed variations in piglet growth and weight which were higher in the treated group. Such observations are in accordance with a recent report showing that the dietary composition of pregnant women can influence the weight of their babies at birth (Moore *et al.* 2004). Piglet birth weight is highly correlated to litter size. In the present study, while litter size in the treated group was lower but not significantly different than in the control group, birth weight was significantly higher in the treated group. Therefore this factor could have influenced weight at weaning and growth, and the role of SLO supplementation could not be clearly identified for these two parameters. Previous studies have found that supplementing sows with tuna oil, an oil rich in *n*-3 PUFA, during pregnancy resulted in improved offspring growth (Rooke *et al.* 2001). However, growth improvement could also at least partially result from the additional energy intake provided by SLO supplementation in addition to heavier birth weight of piglets from the supplemented group. The use of SLO, which associated *n*-3 PUFA with alkyl-Gro, may help to match the nutritional requirements in *n*-3 PUFA of the newborn piglet and improve health status by protecting the offspring from environmental threats.

Among mechanisms that could be involved in such effects, we have shown that alkyl-Gro could serve as precursors of platelet-activating factor and amplify its production in monocyte-like THP-1 cells (Hichami *et al.* 1997). Platelet-activating factor is involved in a wide diversity of pro-inflammatory and immune responses: it acts as an agonist on perforin-induced membrane

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damage by natural killer cells (Berthou *et al.* 2000), and helps transendothelial migration of leucocytes by increasing their surface cell-adhesion molecules (Neeley *et al.* 1993). Platelet-activating factor also induces cytokine liberation by platelets (Lindemann *et al.* 2001) and activates polymorphonuclear cells and monocytes (Zimmerman *et al.* 2002). Moreover, alkyl-Gro also can be incorporated into cell membrane phospholipids and lead to the production of alkylacylglycerol (Marigny *et al.* 2002), an antagonist of diacylglycerol in the protein kinase C activation pathway (Heymans *et al.* 1987; Daniel *et al.* 1988).

We demonstrated herein that dietary supplementation with SLO, a natural source of alkyl-Gro and *n*-3 PUFA, to gestating and lactating sows induced a positive effect on litter immune status associated with a modification of both lipid content and immune properties of the mother's milk. Furthermore, the swine animal model is relevant for bringing to evidence potential interest of such a diet for neonates (Innis, 1993). These observations could be of particular interest for offspring health as well as a nutritional intervention intended for pregnancy and lactation.

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References

Anderson M & Fritsche KL (2002) (*n*-3) Fatty acids and infectious disease resistance. *J Nutr* **132**, 3566–3576.

Bashiri A, Burstein E, Sheiner E & Mazor M (2003) Anemia during pregnancy and treatment with intravenous iron: review of the literature. *Eur J Obstet Gynecol Reprod Biol* **110**, 2–7.

Berthou C, Bourge JF, Zhang Y, Soulie A, Geromin D, Denizot Y, Sigaux F & Sasportes M (2000) Interferon-γ-induced membrane PAF-receptor expression confers tumor cell susceptibility to NK perforin-dependent lysis. *Blood* **95**, 2329–2336.

Bligh EG & Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Med Sci* **37**, 911–917.

Blok WL, Katan MB & van der Meer JW (1996) Modulation of inflammation and cytokine production by dietary (*n*-3) fatty acids. *J Nutr* **126**, 1515–1533.

Bouwstra H, Dijck-Brouwer DA, Wildeman JA, Tjoonk HM, van der Heide JC, Boersma ER, Muskiet FA & Hadders-Algra M (2003) Long-chain polyunsaturated fatty acids have a positive effect on the quality of general movements of healthy term infants. *Am J Clin Nutr* **78**, 313–318.

Brohult A, Brohult J & Brohult S (1972) Effect of irradiation and alkoxyglycerol treatment on the formation of antibodies after salmonella vaccination. *Experientia* **28**, 954–955.

Brohult A, Brohult J & Brohult S (1978) Regression of tumour growth after administration of alkoxyglycerols. *Acta Obstet Gynecol Scand* **57**, 79–83.

Brohult A, Brohult J, Brohult S & Joelsson I (1977) Effect of alkoxyglycerols on the frequency of injuries following radiation therapy for carcinoma of the uterine cervix. *Acta Obstet Gynecol Scand* **56**, 441–448.

Calder PC (1998) Dietary fatty acids and the immune system. *Nutr Rev* **56**, S70–S83.

Cheminade C, Gautier V, Hichami A, Allaume P, Le Lannou D & Legrand AB (2002) 1-*O*-Alkylglycerols improve boar sperm motility and fertility. *Biol Reprod* **66**, 421–428.

Chorostowska-Wynimko J, Krotkiewski M, Radomska-Lesniewska D, Sokolnicka I & Skopinska-Rozewska E (2001) The synergistic effect of lactic acid bacteria and alkylglycerols on humoral immunity in mice. *Int J Tissue React* **23**, 81–87.

Daniel LW, Small GW, Schmitt JD, Marasco CJ, Ishaq K & Piantadosi C (1988) Alkyl-linked diglycerides inhibit protein kinase C activation by diacylglycerols. *Biochem Biophys Res Commun* **151**, 291–297.

Francois CA, Connor SL, Bolewicz LC & Connor WE (2003) Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. *Am J Clin Nutr* **77**, 226–233.

Hallgren B & Larsson S (1962) The glyceryl ethers in man and cow. *J Lipid Res* **3**, 39–43.

Helland IB, Smith L, Saarem K, Saugstad OD & Drevon CA (2003) Maternal supplementation with very-long-chain *n*-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics* **111**, e39–e44.

Heymans F, Da Silva C, Marrec N, Godfroid JJ & Castagna M (1987) Alkyl analogs of diacylglycerol as activators of protein kinase C. *FEBS Lett* **218**, 35–40.

Hichami A, Duroudier V, Leblais V, Vernhet L, Le Goffic F, Ninio E & Legrand A (1997) Modulation of platelet-activating-factor production by incorporation of naturally occurring 1-*O*-alkylglycerols in phospholipids of human leukemic monocyte-like THP-1 cells. *Eur J Biochem* **250**, 242–248.

Hoffman DR, Birch EE, Birch DG, Uauy R, Castaneda YS, Lapus MG & Wheaton DH (2000) Impact of early dietary intake and blood lipid composition of long-chain polyunsaturated fatty acids on later visual development. *J Pediatr Gastroenterol Nutr* **31**, 540–553.

Innis SM (1993) The colostrum-deprived piglet as a model for study of infant lipid nutrition. *J Nutr* **123**, 386–390.

Kitessa SM, Gulati SK, Simos GC, Ashes JR, Scott TW, Fleck E & Wynn PC (2004) Supplementation of grazing dairy cows with rumen-protected tuna oil enriches milk fat with *n*-3 fatty acids without affecting milk production or sensory characteristics. *Br J Nutr* **91**, 271–278.

Kumar R, Weintraub ST & Hanahan DJ (1983) Differential susceptibility of mono- and di-*O*-alkyl ether phosphoglycerides to acetolysis. *J Lipid Res* **24**, 930–937.

Lindemann S, Tolley ND, Dixon DA, McIntyre TM, Prescott SM, Zimmerman GA & Weyrich AS (2001) Activated platelets mediate inflammatory signaling by regulated interleukin 1β synthesis. *J Cell Biol* **154**, 485–490.

Linman JW, Bethell FH & Long MJ (1958) The erythropoietic stimulatory activity of batyl alcohol. *J Lab Clin Med* **52**, 596–604.

Liu Y, Gong L, Li D, Feng Z, Zhao L & Dong T (2003) Effects of fish oil on lymphocyte proliferation, cytokine production and intracellular signalling in weanling pigs. *Arch Tierernahr* **57**, 151–165.

Marigny K, Pédrono F, Martin-Chouly CA, Youmine H, Saiag B & Legrand AB (2002) Modulation of endothelial permeability by 1-*O*-alkylglycerols. *Acta Physiol Scand* **176**, 263–268.

Mitre R, Cheminade C, Allaume P, Legrand P & Legrand A (2004) Oral intake of shark liver oil modifies lipid composition and improves motility and velocity of boar sperm. *Theriogenology* **62**, 1557–1566.

Moore VM, Davies MJ, Willson KJ, Worsley A & Robinson JS (2004) Dietary composition of pregnant women is related to size of the baby at birth. *J Nutr* **134**, 1820–1826.

Neeley SP, Hamann KJ, White SR, Baranowski SL, Burch RA & Leff AR (1993) Selective regulation of expression of surface adhesion molecules Mac-1, L-selectin, and VLA-4 on human eosinophils and neutrophils. *Am J Respir Cell Mol Biol* **8**, 633–639.

Ngwenya BZ & Foster DM (1991) Enhancement of antibody production by lysophosphatidylcholine and alkylglycerol. *Proc Soc Exp Biol Med* **196**, 69–75.

https://doi.org/10.1079/BJN20051569 Published online by Cambridge University Press

Oh SY & Jadhav LS (1994) Effects of dietary alkylglycerols in lactating rats on immune responses in pups. *Pediatr Res* **36**, 300–305.

Pedrono F, Martin B, Leduc C, Le Lan J, Saiag B, Legrand P, Moulinoux JP & Legrand AB (2004) Natural alkylglycerols restrain growth and metastasis of grafted tumors in mice. *Nutr Cancer* **48**, 64–69.

Pugliese PT, Jordan K, Cederberg H & Brohult J (1998) Some biological actions of alkylglycerols from shark liver oil. *J Altern Complement Med* **4**, 87–99.

Rooke JA, Sinclair AG & Edwards SA (2001) Feeding tuna oil to the sow at different times during pregnancy has different effects on piglet long-chain polyunsaturated fatty acid composition at birth and subsequent growth. *Br J Nutr* **86**, 21–30.

Salmon H (1999) The mammary gland and neonate mucosal immunity. *Vet Immunol Immunopathol* **72**, 143–155.

Taugbol O, Framstad T & Saarem K (1993) Supplements of cod liver oil to lactating sows. Influence on milk fatty acid composition and growth performance of piglets. *Zentralbl Veterinarmed A* **40**, 437–443.

Voigt RG, Jensen CL, Fraley JK, Rozelle JC, Brown FR 3rd & Heird WC (2002) Relationship between omega3 long-chain polyunsaturated fatty acid status during early infancy and neurodevelopmental status at 1 year of age. *J Hum Nutr Diet* **15**, 111–120.

Yamamoto N, St Claire DA Jr, Homma S & Ngwenya BZ (1988) Activation of mouse macrophages by alkylglycerols, inflammation products of cancerous tissues. *Cancer Res* **48**, 6044–6049.

Zimmerman GA, McIntyre TM, Prescott SM & Stafforini DM (2002) The platelet-activating factor signaling system and its regulators in syndromes of inflammation and thrombosis. *Crit Care Med* **30**, S294–S301.