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Effect of dietary supplementation with docosahexaenoic acid to sows during lactation and gestation on litter size, piglet birthweight and colostrum composition and plasma concentrations of fatty acids and cytokines

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Abstract

This study investigated the effects of dietary docosahexaenoic acid (DHA) supplementation in sows on blood fatty acid and cytokine concentrations during late gestation, piglet birth weight and within-litter variation, and colostrum composition. Multiparous sows (n = 227) were fed either a standard diet or a diet supplemented with 50 mg DHA/kg from farrowing of the previous litter until farrowing of the subsequent litter. The numbers of liveborn and stillborn piglets and individual piglet birth weights were recorded. In a subsample of 46 sows, blood samples collected from the jugular vein six days before farrowing were analyzed for cytokine and glucose concentrations. Colostrum samples from 19 sows, collected 12 h after farrowing, were analyzed for nutrient composition, immunoglobulin concentrations, and fatty acid profile. Dietary DHA supplementation did not affect the numbers of liveborn or stillborn piglets, individual piglet birth weight, or within-litter variation in birth weight. Plasma cytokine concentrations also did not differ between treatments, whereas plasma glucose concentrations were higher in DHA-supplemented sows. Colostrum composition was largely unaffected by treatment, although DHA concentration tended to be greater in colostrum from supplemented sows. In conclusion, supplementing sow diets with 50 mg DHA/kg throughout an entire reproductive cycle had no measurable effects on piglet birth outcomes, colostrum composition, or plasma cytokine concentrations, but increased plasma glucose concentrations in late gestation.

Keywords: Colostrum composition, Omega-3 fatty acid, Piglet birth weight, Plasma cytokines, Plasma glucose, Sow, Within-litter variation.

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Contents

Contribution of this paper to the literature

This study contributes to the existing literature by examining the impact of feeding a low dose of omega-3 fatty acid to sows throughout the entire reproductive cycle. It offers insight into whether a low dose of dietary omega-3 fatty acids is enough to see an effect on the productivity and health of the sows.

1. Introduction

The increasing litter size of hyper-prolific sows has been associated with reduced individual piglet birth weight and a greater incidence of intrauterine growth-retarded (IUGR) piglets within litters [1], both of which contribute to increased pre-weaning mortality [2]. Docosahexaenoic acid (DHA) is one of the most important n-3 fatty acids. Fish oils and microalgae are rich sources of DHA, whereas vegetable-derived dietary fat sources contain little or no DHA. Standard European sow diets, typically based on barley, wheat, soybean meal, and soybean oil, are naturally rich in n-6 fatty acids, particularly linoleic acid (LA). Dietary fatty acids such as LA and α-linolenic acid (ALA) compete for the same elongase and desaturase enzymes during metabolism, leading to the synthesis of arachidonic acid and DHA, respectively [3]. Consequently, providing DHA directly through the diet may be more effective than relying on endogenous conversion from ALA.

In the present study, a microalgae product previously evaluated by Adeleye, et al. [4] was used as the DHA source. In that study, sows received either 300 or 3000 mg DHA/kg feed from mid-gestation through lactation. Both supplementation levels reduced the number of stillborn piglets and improved piglet vitality. However, DHA supplementation also prolonged farrowing duration, and piglet weaning weight was reduced at the higher dosage of 3000 mg/kg feed [4]. To minimize potential adverse effects while maintaining potential benefits, a lower supplementation level of 50 mg DHA/kg feed was selected for the present study.

Maternal dietary n-3 fatty acids are transferred to the fetus during gestation [5].

The mammalian brain and retina contain high concentrations of DHA and arachidonic acid [6] and both prenatal and postnatal development of the brain, visual system, and nervous system depend on an adequate supply of n-3 fatty acids [7]. Therefore, maternal transfer of n-3 fatty acids during gestation may play an important role in neonatal survival and growth.

Excessive inflammation during pregnancy has been associated with miscarriage, preterm birth, and IUGR in humans [8]. Studies in both humans and rodents have demonstrated that n-3 fatty acids can reduce the production of pro-inflammatory cytokines [9], while maternal supplementation with n-3 fatty acids has been linked to increased fetal and placental growth in rats [10]. Docosahexaenoic acid (DHA) inhibits the synthesis of n-6-derived prostaglandins, thereby promoting a less inflammatory uterine environment [11], which may improve embryo and fetal survival. Furthermore, dietary supplementation with n-3 fatty acids has been shown to increase litter size [12], potentially through beneficial alterations of the uterine environment, as suggested by Gokuldas, et al. [13].

Several studies have demonstrated that dietary omega-3 fatty acids are incorporated into sow colostrum [14-16]. However, changes in colostrum fatty acid composition do not necessarily translate into improved litter growth [14, 16], although they may confer other benefits, such as enhanced resistance to inflammation in newborn piglets. In humans, increased dietary DHA intake results in higher DHA concentrations in colostrum and milk [17]. Similarly, a study in dairy cattle reported increased colostral immunoglobulin concentrations following DHA supplementation [18].

The response to dietary n-3 fatty acid supplementation in sows depends on both supplementation duration and dosage. Supplementation with a high dose of DHA (4.03 g/kg feed) during the first 45 days of gestation reduced the number of piglets weighing less than 800 g at birth (2.3 vs. 1.8 piglets per litter). However, neither average birth weight nor within-litter variation in birth weight was affected, suggesting that the supplementation period was too short to influence fetal growth. Likewise, Lyderik, et al. [19] reported that differences in fetal and placental weights within litters were already evident by day 28 of gestation. These findings suggest that short-term dietary interventions initiated during mid- or late gestation may be insufficient to influence birth weight and that supplementation should commence before mating. Several studies have therefore investigated omega-3 fatty acid supplementation throughout gestation or the entire reproductive cycle to improve litter size and piglet birth weight [20, 21]. To optimize the uterine environment through reduced inflammation and altered gene expression, supplementation may need to begin before fertilization, as dietary anti-inflammatory effects are unlikely to occur immediately. In support of this concept, dietary omega-3 fatty acids have also been shown to influence follicular development in gilts [22]. Furthermore, continuous supplementation throughout the reproductive cycle may be necessary to maintain these effects.

We hypothesized that supplementing sow diets with a low dose of DHA throughout the entire reproductive cycle would improve litter size, enhance placental function, and consequently increase piglet birth weight. Therefore, the objective of this study was to evaluate the effects of low-level DHA supplementation on litter size, piglet birth weight and within-litter variation, colostrum composition and fatty acid profile, and cytokine concentrations in sow blood.

2. Materials and Methods

2.1. Animals and Housing

A total of 227 multiparous hybrid sows (Landrace × Yorkshire; DanBred) inseminated with Duroc semen (DanBred, Ornestation Mors, Redsted, Denmark) were included in this study, which was conducted in a commercial Danish sow herd. The parity distribution of the sows was 25% parity 2, 22% parity 3, 20% parity 4, 19% parity 5, and 14% parity 6. During gestation, the sows were housed in loose groups of 70–80 animals per pen. Approximately seven days before the expected farrowing date, the sows were moved to the farrowing unit, where they were individually housed in farrowing crates. Each pen was equipped with a covered creep area for piglets and an infrared heating lamp (VengSystem A/S, Roslev, Denmark), which gradually reduced the temperature after farrowing. Artificial lighting was provided from 06:30 to 16:00 h in all units, although natural daylight was also available through windows. During the main farrowing period, the herd was monitored between 19:00 and 01:00 h, with inspections conducted every 30–60 min. If no piglets had been born since the previous inspection, farrowing

assistance was provided. Standard sow and piglet management procedures were applied according to routine herd practices, and all procedures were carried out by permanent herd staff. Each week, a batch of 18 sows was randomly selected and allocated to the experiment, stratified by parity, upon transfer to the farrowing unit. This procedure ensured a similar average parity distribution between the two experimental groups.

2.2. Diets and Feeding System

Sow diets were mixed using a SpotMix feeding system (Schauer Agrotronic GmbH, Prambachkirchen, Austria), which also weighed and distributed the individual feed rations. The SpotMix system uses air-supported transport for feed distribution, ensuring that no detectable feed residues remain in the pipes and thereby minimizing the risk of DHA cross-contamination between treatment groups. A common feeding curve was applied to all sows in the farrowing unit. The maximum feed allowance was set slightly above the expected voluntary feed intake to ensure that any differences observed in the measured parameters could be attributed solely to the dietary treatment. Before farrowing, sows were offered 3.1 kg feed/day. During the first three days after farrowing, feed allowance was gradually increased until reaching a maximum of 8.5 kg/day at 21 days postpartum. From weaning until mating, feed allowance was gradually reduced from 5.5 to 3.2 kg/day. During gestation, sows were fed individually through electronic sow feeders (Compident 8, Schauer Agrotronic GmbH, Prambachkirchen, Austria) according to body condition. Thin, normal, and fat sows received 3.8, 3.1, and 2.8 kg feed/day, respectively, from days 1 to 28 of gestation. From days 29 to 84, normal and fat sows received 2.2 kg/day, whereas thin sows received 2.7 kg/day. From days 85 to 114 of gestation, normal and fat sows were offered 3.1 kg/day, while thin sows received 3.4 kg/day.

In the gestation unit, the 24-h feeding period began at 22:00 h, and sows were allowed to consume their entire daily ration in a single meal. In the farrowing unit, sows were fed three equal meals per day at approximately the same times each day: between 04:30 and 09:30 h, 11:30 and 16:30 h, and 19:30 and 00:30 h. In both the gestation and farrowing units, sows had ad libitum access to water via drinking nipples.

On the day of the previous farrowing, sows were allocated to one of two dietary treatments: a control diet or a diet supplemented with a DHA source derived from microalgae (declared C22:6 n-3 content > 17%; DSM-Firmenich Animal Nutrition & Health, Basel, Switzerland). The dietary DHA concentration of 50 mg/kg feed was selected based on the study by Adeleye, et al. [4], in which sows were supplemented with either 300 or 3000 mg DHA/kg feed. Both supplementation levels reduced the number of stillborn piglets and improved piglet vitality. However, both levels also increased farrowing duration, and the higher supplementation level reduced piglet weaning weight. To minimize these undesirable effects while retaining potential benefits, the recommended DHA supplementation level of 50 mg/kg feed was used in the present study. All experimental diets had the same basal composition (Table 1), with the only difference being the inclusion of DHA in the supplemented diets. In total, ten diets were used: five control diets and five DHA-supplemented diets. These diets corresponded to five feeding phases: the weaning-to-service period (diet 1), early gestation (days 0–84; diet 2), late gestation (days 85–110; diet 3), the transition period from day 111 of gestation to day 3 of lactation (diet 4), and lactation from day 4 postpartum until weaning at day 28 (diet 5). The basal diet was formulated using soybean meal, barley, wheat, dried sugar beet pulp, and soybean oil, with vitamin and mineral premixes added according to the stage of gestation or lactation (Table 1). Feed samples were collected from each of the ten diets throughout the experimental period (6–10 samples per diet) and pooled to obtain three to four composite samples per diet for chemical analysis.

Table 1. Feed compositions of the control group in different periods of the reproductive cycle. Compositions were the same in the DHA group, except for addition of 50 mg DHA per kg feed[1].

Diet[2]	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Ingredients, %					
Wheat	51.60	15.00	15.00	27.30	38.00
Spring barley	30.00	63.90	62.70	45.00	35.00
Dried sugar beet pulp	2.00	8.00	5.00	5.00	2.50
Soybean meal, dehulled	10.10	8.60	11.80	15.90	17.60
Soy oil	1.50	0.50	1.50	2.00	2.10
Monocalcium phosphate	1.05	0.75	0.75	1.05	1.05
Limestone	1.33	1.39	1.39	1.33	1.33
Sodium chloride	0.58	0.62	0.62	0.58	0.58
L-Lysine	0.31	-	-	0.31	0.31
DL-Methionine	0.08	-	-	0.08	0.08
L-Threonine	0.12	-	-	0.12	0.12
L-Valine	0.02	-	-	0.02	0.02
Mineral premix gestating[3]	-	1.24	1.24	-	-
Mineral premix lactating[4]	1.31	-	-	1.31	1.31
Calculated chemical composition, g/kg					
Dry matter	862	855	855	862	863
Crude protein	123	112	123	143	150
Crude fat	36	27	37	41	42
Ash	51	55	56	56	55
Energy (MJ ME per kg)[5]	13.24	12.63	12.91	13.17	13.34
Energy (MJ NE per kg)[5]	10.14	9.52	9.76	9.91	10.06
Calculated amino acid content (g SID per kg)					
Lysine	6.87	4.20	4.96	8.28	8.72
Methionine	2.41	1.49	1.64	2.66	2.76
Methionine + cysteine	4.36	3.27	3.58	4.78	5.01
Threonine	4.57	3.13	3.61	5.36	7.85
Calculated mineral content (g per kg)					
Phosphorus	3.40	2.67	2.78	3.51	3.60
Calcium	7.55	6.44	6.41	7.88	7.85

Note: [1] The only difference between the control and experimental feed was that 50 mg C22:6 n-3 (DHA) was added to the mineral premixed for experimental feed for both lactating and pregnant sows in the form of 300 mg DHA Gold (declared content of C22:6 n-3> 17%, CP = 17%, crude fat = 56%, ash = 8.8%, crude fiber = 4.5%, carbohydrate = 12.4%; dsm-firmenich Animal Nutrition & Health, Basel, Switzerland).
[2]Diet 1: Used during service. Diet 2: Day 0 to day 84 of gestation. Diet 3: Day 85 to day 110 of gestation. Diet 4: Day 111 of gestation to day 3 of lactation. Diet 5: Day 4 of lactation to weaning.

[3]Mineral premix for gestating sows provided the following amounts of minerals and vitamins per kg diet: 9,900 IU vitamin A; 900 IU vitamin D3; 22.5 µg 25-hydroxy vitamin D3 (Hy-D, dsm-firmenich Animal Nutrition & Health, Basel, Switzerland); 199 mg DL-alpha-tocopherol; 4.0 mg vitamin K3 (menadione); 2.0 mg vitamin B1; 5.0 mg vitamin B2; 3.0 mg vitamin B6; 0.02 mg vitamin B12; 1.9 mg folic acid; 19.9 mg niacin; 14.9 mg D-pantothenic acid; 49.6 mg iron ($FeSO_4$); 49.6 mg iron fumerate; 9.9 mg copper ($CuSO_4$); 5.0 mg organic copper (copper(II) chelate of glycine hydrate, B-TRAXIM 2C, Pancosma, Rolle, Switzerland); 26.8 mg manganese (MnO); 12.9 mg organic manganese (manganese chelate of glycine hydrate, B-TRAXIM 2C, Pancosma, Rolle, Switzerland); 66.5 mg zinc (ZnO); 32.8 mg organic zinc (zinc chelate of glycine hydrate, B-TRAXIM 2C, Pancosma, Rolle, Switzerland); 0.5 mg; iodine ($Ca(IO_3)$; 0.20 mg selenium (Na_2SeO_3); 0.15 mg organic selenium (ALKOSEL^{R397}3000, Lallemand, Blagnac, France); 248 mg betainhydrochloride, 1×10^9 CFU live yeast (*Saccharomyces cerevisiæ* CNCM I-1079, LEVUCELL® SB 10ME, Lallemand, Blagnac, France); 5000 mg benzoic acid; 1,500 phytase activity units (Ronozyme® HiPhos, dsm-firmenich Animal Nutrition & Health, Basel, Switzerland).

[4] Mineral premix for lactating sows provided the following amounts of minerals and vitamins per kg diet: 10,800 IU vitamin A; 900 IU vitamin D3; 22.5 µg 25-hydroxy vitamin D3 (Hy-D, dsm-firmenich Animal Nutrition & Health, Basel, Switzerland); 217 mg DL-alpha-tocopherol; 4.3 mg vitamin K3 (menadione); 2.4 mg vitamin B1; 5.4 mg vitamin B2; 3.2 mg vitamin B6; 0.02 mg vitamin B12; 2.3 mg folic acid; 21.7 mg niacin; 16.2 mg D-pantothenic acid; 54.1 mg iron ($FeSO_4$); 54.1 mg iron fumerate; 10.8 mg copper ($CuSO_4$); 5.4 mg organic copper (copper(II) chelate of glycine hydrate, B-TRAXIM 2C, Pancosma, Rolle, Switzerland); 29.2 mg manganese (MnO); 14.1 mg organic manganese (manganese chelate of glycine hydrate, B-TRAXIM 2C, Pancosma, Rolle, Switzerland); 72.5 mg zinc (ZnO); 35.7 mg organic zinc (zinc chelate of glycine hydrate, B-TRAXIM 2C, Pancosma, Rolle, Switzerland); 0.5 mg; iodine ($Ca(IO_3)$; 0.22 mg selenium (Na_2SeO_3); 0.16 mg organic selenium (ALKOSEL^{R397}3000, Lallemand, Blagnac, France); 271 mg betainhydrochloride, 1×10^9 CFU live yeast (*Saccharomyces cerevisiæ* CNCM I-1079, LEVUCELL® SB 10ME, Lallemand, Blagnac, France); 7000 mg benzoic acid; 1,500 phytase activity units (Ronozyme® HiPhos, dsm-firmenich Animal Nutrition & Health, Basel, Switzerland).

[5]Calculated using EvaPig.

The dietary composition of feed samples was analyzed at Eurofins Steins Laboratory A/S (Vejen, Denmark) in accordance with the European Commission Directives [EC] 64/1998 and [EC] 152/2009. The fatty acid (FA) profile of the diet was determined by extracting dietary fat using a modified Bligh and Dyer method, as described by Jensen [23], followed by gas-liquid chromatography (Hewlett Packard 6890, Agilent Technologies, Palo Alto, CA, USA) to analyze the fatty acid composition.

2.3. Data Collection, Measurements, and Chemical Analyses

On the day of farrowing, the numbers of live-born and stillborn piglets were recorded for all sows. Individual piglet birth weights were measured within 30 min of birth using a wagon scale with a precision of 10 g (Bjerringbro Vægte ApS, Bjerringbro, Denmark). Blood (n = 45) and colostrum (n = 19) samples were collected from a subsample of second- and third-parity sows. Blood samples were obtained 6.3 ± 1.1 days before farrowing and approximately 3 h after the morning feeding. Blood was collected from the jugular vein by venipuncture into 10-mL EDTA vacutainer tubes (BD Vacutainer®, KRUUSE, Langeskov, Denmark). Samples were kept on ice until centrifugation at 1,558 × g for 10 min at room temperature. Plasma was then harvested and stored at −20°C until analysis. Plasma concentrations of interleukin-10 (IL-10), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α) were determined using porcine-specific DuoSet ELISA kits according to the manufacturer's instructions (R&D Systems, Abingdon, UK). Colostrum samples were collected 12 h after the birth of the first piglet. Samples were filtered through a double layer of gauze and stored at −20°C until analysis. Colostrum composition, including dry matter, lactose, protein, and fat content, was determined by infrared spectroscopy (MilkoScan FT2, FOSS Electric, Hillerød, Denmark). Brix values were measured using a digital refractometer (PAL-1, Atago, Saitama, Japan). The fatty acid (FA) profile of the colostrum was analyzed using the same method as that employed for the feed samples.

2.4. Calculations and Statistical Analysis

For each litter, the average birth weight of all piglets, the average birth weight of liveborn piglets, and the average birth weight of stillborn piglets were calculated. Additionally, the proportion of liveborn piglets with birth weights below 800 g, 1,000 g, and above 1,600 g, and 1,800 g was calculated for each litter. Calculations and statistical analyses were performed using the statistical software R (version 4.2.1, R Core Team, Vienna, Austria). The sow or litter was considered the experimental unit. When analyzing litter data, averages for each sow based on individual piglet birth weights within the litter were used. Litter averages included both live and stillborn piglets. The normality of the data and variance homogeneity were confirmed. Data were fitted using a mixed-effects model in ANOVA form using the nlme package in R to examine the effects of diet and parity. The effect of dietary treatment on the number of stillborn piglets was analyzed using a logistic regression model assuming a binomial distribution. The effect of dietary treatment on the proportion of piglets with birth weights below 800 g, 1,000 g, and the proportion of piglets weighing above 1,600 g, and 1,800 g was analyzed using a logistic regression model. Models included batch of sows as a random effect, and diet and parity as fixed effects. The interaction between diet and parity was also included but was removed from the model when found to be non-significant. The number of total born piglets was included as a covariate when analyzing litter and colostrum composition data, but was removed from the model when found to be non-significant. Results are presented as least-squares means with standard errors. When necessary, data were log-transformed to conform to normality. The log-transformed data were presented as least-squares means with a 95% confidence interval (CI). Pearson correlation coefficients were used to examine the relationship between the day of blood sampling, plasma concentrations of IL-6, IL-10, TNF-α, glucose, and litter characteristics at farrowing. Statistical significance was declared at $P<0.05$, with tendencies noted for $0.05<P<0.10$.

3. Results

3.1. Diets

Apart from fatty acid (FA) composition, the nutrient concentrations of the control and DHA-supplemented diets were similar (Suppl. Table 1). As expected, DHA concentrations were greater ($P < 0.05$) in all DHA-supplemented diets (29–68 mg/kg) than in the corresponding control diets (0–3 mg/kg). Despite these differences in DHA content, total n-6 FA concentration, total n-3 FA concentration, and the n-6:n-3 FA ratio did not differ between dietary treatments ($P > 0.05$, Suppl. Table 2).

3.2. Plasma Cytokines and Glucose

Dietary treatment did not affect plasma concentrations of IL-6, IL-10, or TNF-α. However, third-parity sows had lower plasma IL-6 concentrations (3,103 vs. 9,799 pg/mL) and higher plasma IL-10 concentrations (202 vs. 118 pg/mL) than second-parity sows. In contrast to the cytokine results, plasma glucose concentrations were greater in DHA-supplemented sows than in control sows (Table 2).

Table 2. Sow plasma concentrations of cytokines (IL-6, IL-10 and TNFα) and glucose measured at d 6 pre-partum in a subsample of sows.

Item	Control	DHA	SEM	P-value	
				Diet	Parity
Number of sows	22	23			
Parity	2.55	2.48	0.11	0.66	-
TNF-α, pg/mL	757	679	[321; 1,422][1]	0.72	0.34
IL-6, pg/mL	5,324	5,768	[2,080; 15,522][1]	0.56	<0.001
IL-10, pg/mL	149	161	[92.8; 262][1]	0.82	<0.01
Glucose, mmol/L	3.57	4.00	0.21	0.03	0.16

Note: [1] CI = Confidence Interval (95%).

3.3. Farrowing Results

The proportion of piglets weighing >1,600 g at birth was greater in the control group than in the DHA group ($P < 0.05$), and a similar tendency was observed for piglets weighing >1,800 g ($P < 0.10$). Neither litter size nor average piglet birth weight was affected by dietary treatment.

Parity affected litter birth weight, which was greater in parity 3 than in parity 6 sows. Parity 6 sows also had a higher proportion of piglets weighing <1,000 g at birth than parity 3 and 4 sows. Conversely, parity 3 sows had a higher proportion of piglets weighing >1,600 g, and parity 3 and 4 sows had a higher proportion of piglets weighing >1,800 g than the other parity groups ($P < 0.01$). The numbers of total born and liveborn piglets were similar across parities (Table 3; Figure 1).

Table 3. Effect of feeding a control or DHA supplemented diet during lactating and gestation on litter characteristics at farrowing and individual piglet birthweight.

Item	Control	DHA	SEM	P-value	
				Diet	Parity
n sows	110	117			
Parity	3.7	3.8			
Total born piglets	19.75	19.67	0.43	0.88	0.13
Liveborn piglets	18.55	18.44	0.37	0.81	0.15
Stillborn piglets, % of total born	5.50	5.91	0.75	0.55	<0.001
Litter weight, kg	26.2	25.7	0.45	0.23	<0.05
Average birthweight, kg	1.35	1.33	0.05	0.41	0.09
Average birthweight live born, kg	1.36	1.34	0.03	0.36	0.14
Average birthweight stillborn, kg	1.09	1.16	0.04	0.27	0.20
Liveborn with BW below 800g, %	5.89	6.53	0.62	0.38	<0.01
Liveborn with BW below 1000g, %	14.9	16.5	1.54	0.14	<0.001
Liveborn with BW above 1600g, %	0.22	0.19	0.03	0.03	<0.001
Liveborn with BW above 1800g, %	0.09	0.07	0.01	0.09	<0.001
Within-litter variation in birth weight, g	293	293	6.54	0.96	0.27
Within-litter CV in birth weight, %	22.2	22.4	0.53	0.75	<0.05

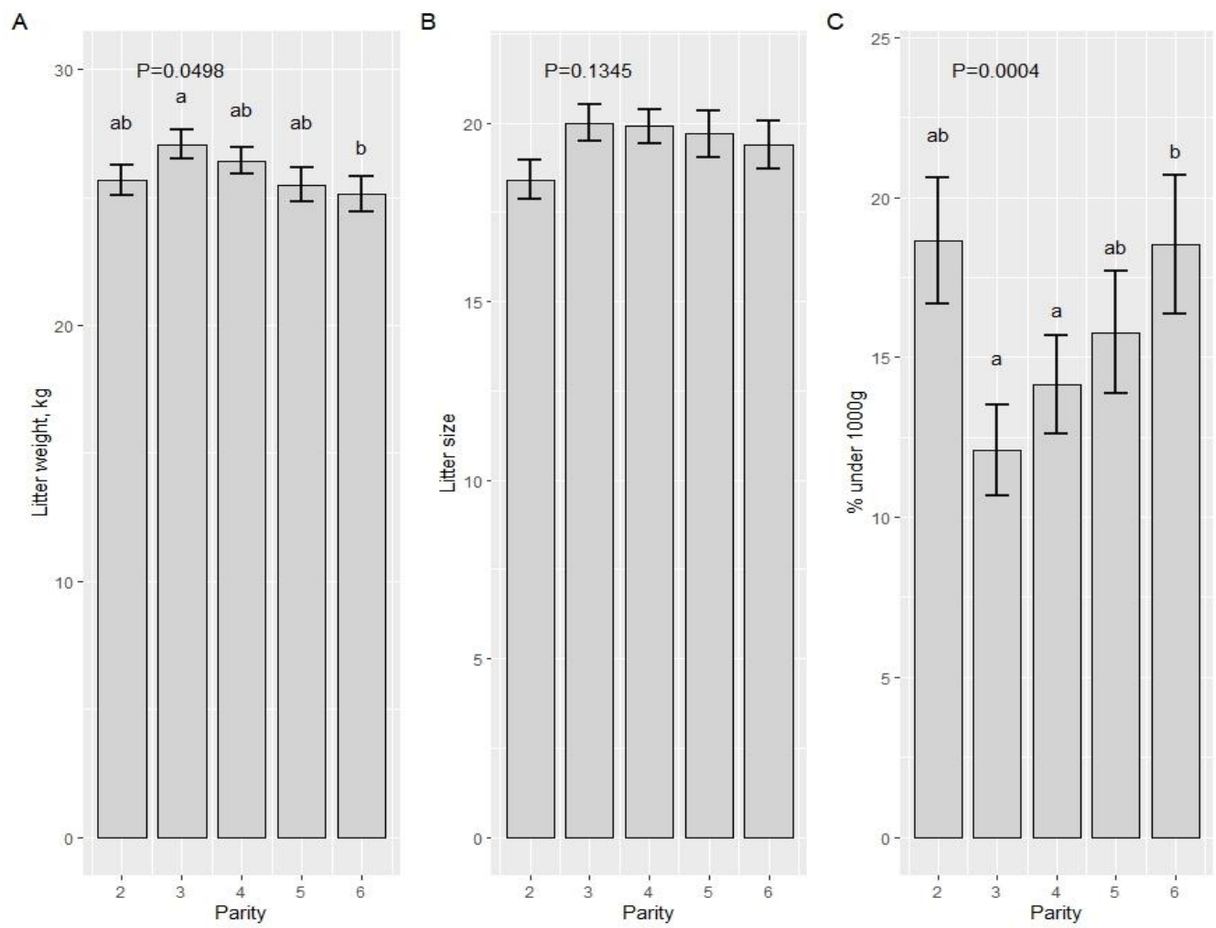

Figure 1. The effect of parity on A) Litter weight at birth, B) Litter size, and C) Percentage of piglets with birth weight below 1000g.

3.4. Colostrum Composition

There was no effect of dietary treatment on the concentrations of macronutrients or immunoglobulins in colostrum (Table 4). The fatty acid composition of colostrum was similar between the groups, with the exception of a tendency for a higher concentration of DHA in colostrum from sows fed the DHA diet (Table 5).

Table 4. Effect of feeding a control or DHA supplemented diet during lactating and gestation on concentrations of macronutrients and immunoglobulins (Ig) in colostrum from a subsample of sows.

Item	Control	DHA	SEM	P-value	
				Diet	Parity
Number of sows	8	11			
Parity	2.62	2.64		0.96	-
Colostrum					
Dry matter, %	22.1	22.3	0.86	0.89	0.54
Fat,%	7.70	7.32	0.84	0.66	0.41
Protein, %	9.75	10.05	0.93	0.84	0.19
Lactose, %	3.39	3.25	0.24	0.69	0.19
Casein, %	7.66	7.88	0.89	0.86	0.26
IgG, mg/mL	31.39	36.16	6.93	0.58	0.11
IgM, mg/mL	1.32	1.53	0.22	0.45	0.87
IgA, mg/mL	8.58	7.88	1.49	0.71	0.11
Brix-value	19.6	19.7	1.19	0.98	0.21

Table 5. Effect of feeding a control or DHA supplemented (50 mg/kg) diet during lactation and gestation on concentrations of nutrients in colostrum.

Fatty acid, mg/ 100 g milk	Control	DHA	SEM	P-value	
				Diet	Parity
Myristic acid (C14:0)	83.6	92.6	16.1	0.62	0.30
Pentadecyclic acid (C15:0)	8.7	9.1	0.98	0.74	0.54
Palmitic acid (C16:0)	1,356	1,356	176	0.98	0.36
Hypogeic acid (C16:1 n9)	62.2	61.8	6.83	0.96	0.72
Palmitoleic acid (C16:1 n-7)	184	179	47.0	0.93	0.13
Heptadecenoic acid (C17:1)	20.6	22.5	2.21	0.45	0.72
Stearic acid (C18:0)	419	408	45.3	0.82	0.44
Oleic acid (C18:1 n-9)	2,233	2,121	332	0.76	0.28
Linoleic acid (C18:2 n-6)	1,286	1,216	117	0.64	0.45
γ-linolenic acid (C18:3 n-6)	24.0	20.6	3.49	0.47	0.42
α-linolenic acid (C18:3 n-3)	107	102	11.03	0.70	0.41
Stearidonic acid (C18:4 n3)	6.24	5.45	0.81	0.44	0.34
Arachidic acid (C20:0)	8.94	8.78	0.89	0.86	0.56
Gondoic acid (C20:1 n-9)	21.5	19.4	3.23	0.60	0.34
Eicosadienoic acid (C20:2 n-6)	27.7	26.4	2.69	0.71	0.37
Dihomo-γ-linolenic acid (C20:3 n-6)	14.2	14.7	1.53	0.80	0.79
Arachidonic acid (C20:4 n-6)	66.6	65.0	6.78	0.86	0.69
Eicosapentaenoic acid (C20:5 n-3)	6.74	6.86	0.75	0.90	0.48
Eicosatrienoic acid (C20:3 n-3)	2.56	2.62	0.27	0.84	0.66
Docosapentaenoic acid (C22:5 n6)	9.92	9.54	1.03	0.78	0.81
Docosapentaenoic acid (C22:5 n-3)	25.6	25.4	2.41	0.96	0.98
Docosahexaenoic acid (C22:6 n-3)	3.87	4.95	0.47	0.09	0.84
Other fatty acids	30.8	32.1	4.58	0.83	0.42
Total n-3 fatty acids	153	148	14.2	0.78	0.51
Total n-6 fatty acids	1,430	1,354	129	0.65	0.46
Ratio n-6: n-3	9.35	9.20	0.11	0.31	0.86

3.5. Correlations

The plasma glucose, IL-6, and TNF-α concentrations were not correlated with litter characteristics at farrowing (P>0.05). Lower plasma concentrations of IL-10 were associated with a higher percentage of piglets weighing below 800 g at birth (r = -0.32; P<0.05) and 1,000 g at birth (r = -0.33; P<0.05). Conversely, higher plasma concentrations of IL-10 were positively correlated with the average piglet birthweight (r = 0.38; P<0.05) but did not correlate with the within-litter variation in birthweight (r = -0.02; P = 0.92). Plasma cytokine concentrations on day 6 pre-partum were not correlated with the milk nutrient or immunoglobulin concentrations in colostrum (P>0.05). The plasma glucose concentration on day 6 pre-partum was positively correlated with protein (r = 0.53; P<0.05), casein (r = 0.53; P<0.05), IgG (r = 0.65; P<0.01), and IgM (r = 0.59; P<0.05) concentrations in colostrum, while it was negatively correlated with the lactose concentration (r = -0.65; P<0.01). Figure 2 illustrates the correlations between plasma cytokines and glucose concentrations, milk nutrient concentrations and farrowing results.

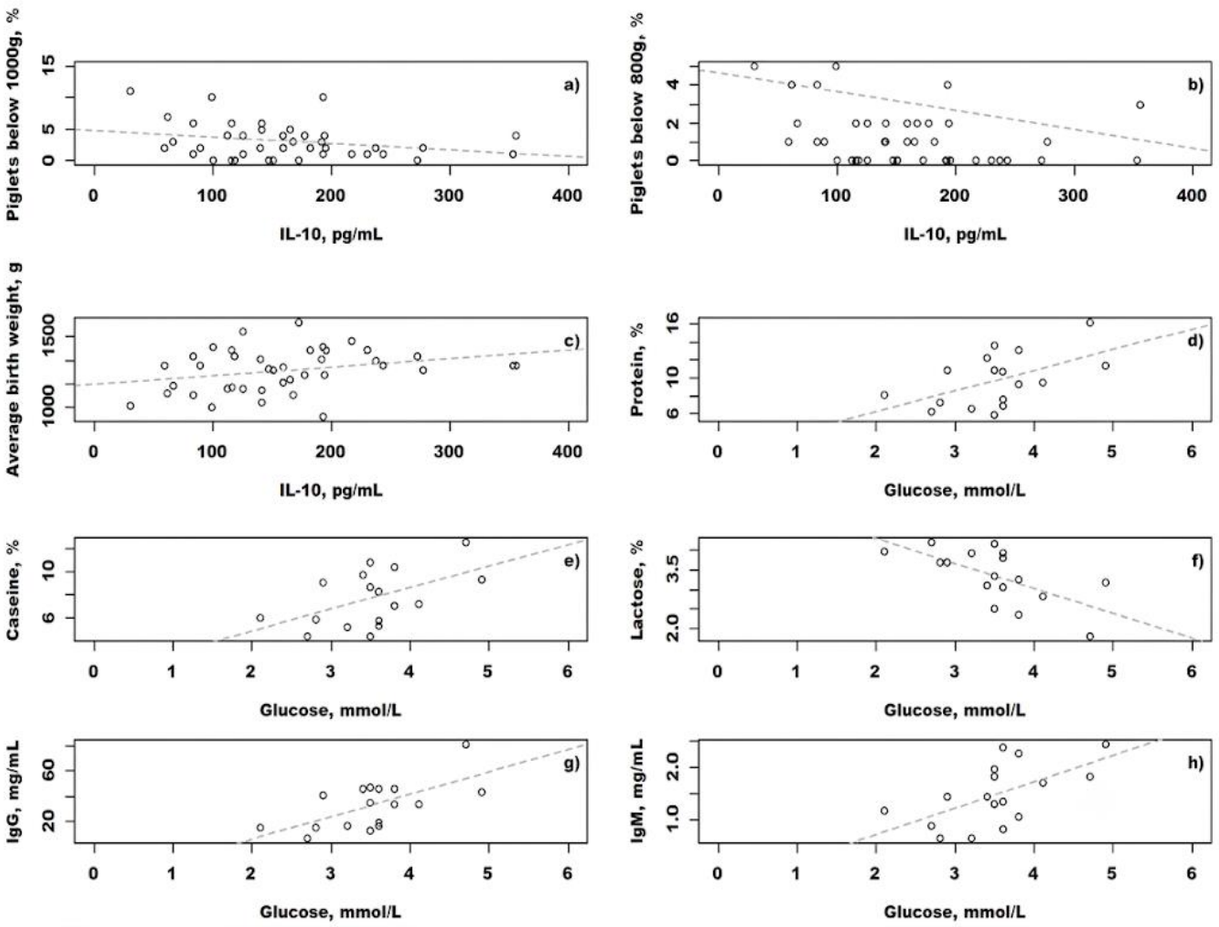

Figure 2. Pearson correlations between plasma and milk concentrations showed a) Lower plasma concentrations of IL-10 were associated with a higher percentage of piglets weighing below 800 g at birth ($r = -0.32$; $P<0.05$), b) Lower plasma concentrations of IL-10 were associated with a higher percentage of piglets weighing 1,000 g at birth ($r = -0.33$; $P<0.05$), c) Higher plasma concentrations of IL-10 were positively correlated with the average piglet birthweight ($r = 0.38$; $P<0.05$), d) The plasma glucose concentration on day 6 pre-partum was positively correlated with milk protein ($r = 0.53$; $P<0.05$), e) The plasma glucose concentration on day 6 pre-partum was positively correlated with milk casein ($r = 0.53$; $P<0.05$), f) The plasma glucose concentration on day 6 pre-partum was negatively correlated with the milk lactose concentration ($r = -0.65$; $P<0.01$), g) The plasma glucose concentration on day 6 pre-partum was positively correlated with milk IgG ($r = 0.65$; $P<0.01$), h) The plasma glucose concentration on day 6 pre-partum was positively correlated with milk IgM ($r = 0.59$; $P<0.05$).

4. Discussion

The objective of the present study was to determine whether long-term supplementation of sow diets with a low level of DHA could improve piglet birth weight and enhance colostrum quality. The experimental diets differed only in the inclusion of a DHA-rich microalgae product, providing 50 mg DHA/kg feed in the supplemented treatment. Consequently, the overall nutrient composition of the diets remained comparable between treatments. Given the relatively low DHA inclusion level, only a small numerical difference in the dietary n-6:n-3 fatty acid ratio was observed between the control and DHA-supplemented diets.

Sows typically develop a degree of insulin resistance from approximately week 12 of gestation onwards, which may impair glucose transport from the maternal circulation to peripheral tissues and across the placenta to the fetuses [24]. Therefore, the higher plasma glucose concentration observed during late gestation in sows fed the DHA-supplemented diet was unexpected. Previous studies have reported that fish oil supplementation can improve insulin sensitivity during pregnancy in both women [25] and sows [26], which would generally be expected to improve blood glucose regulation. However, the sow study found no corresponding effect on piglet birth weight [26].

In contrast, a study in diabetic humans reported increased blood glucose concentrations following omega-3 fatty acid supplementation, although the underlying mechanism was not fully elucidated [27].

Interestingly, in the present study, higher plasma glucose concentrations measured during late gestation were associated with greater colostrum protein concentrations. Elevated glucose availability may stimulate insulin secretion and thereby enhance amino acid uptake by the mammary gland, ultimately promoting protein synthesis [28]. Because the sow tends to prioritize protein deposition in colostrum, including both amino acids and immunoglobulins, this may explain the observed relationship. Conversely, the lower lactose concentration observed in colostrum from sows with higher plasma glucose concentrations may indicate reduced mammary uptake of glucose for lactose synthesis, as lactose is generally a less prioritized component of sow colostrum [29].

The higher plasma glucose concentrations observed in DHA-supplemented sows may also be related to the numerically lower TNF-α concentrations in this group. Tumour necrosis factor-α has been shown to inhibit glucose uptake by tissues, thereby increasing circulating glucose concentrations, as demonstrated in human cell culture studies [30]. Nevertheless, plasma glucose concentrations in both treatment groups were within, or only slightly below, the range previously reported for late-gestating sows [31-33], suggesting that the observed differences were unlikely to have adverse physiological consequences.

To our knowledge, no previous studies have examined the effects of dietary DHA supplementation on plasma cytokine concentrations in sows. Although DHA has been reported to stimulate IL-10 production, dietary treatment had no effect on plasma concentrations of IL-10, IL-6, or TNF-α in the present study. This finding is consistent with a study in piglets in which consumption of colostrum and milk with a lower dietary n-6:n-3 fatty acid ratio (6:1 vs.

10:1) reduced IL-1β concentrations without affecting IL-6 or TNF-α concentrations [34]. Similarly, supplementation of sow diets with 2% fish oil had no effect on maternal plasma IL-6 or TNF-α concentrations [26].

Collectively, these findings suggest that higher inclusion levels of DHA or other n-3 polyunsaturated fatty acids (PUFAs) may be required to induce measurable changes in circulating cytokine concentrations.

Older sows are generally more efficient at transferring passive immunity to their offspring. For example, piglets born to gilts have a greater incidence of neonatal diarrhea [35], and gilts may be more susceptible to oxidative stress and inflammation. This was also evident in the current study, where third parity sows had lower IL-6 and higher IL-10 concentrations compared to second parity sows. Furthermore, parity-3 sows had the lowest proportion of piglets weighing less than 1,000 g at birth. Lower IL-10 concentrations were associated with a higher proportion of low-birth-weight piglets and lower average birth weight. Previous studies by Lavery, et al. [36] and Segura, et al. [37] similarly reported lower average birth weights in first-parity sows than in older sows. Younger sows have also been shown to experience greater oxidative stress [38], which may elevate IL-6 and TNF-α These pro-inflammatory cytokines could negatively influence fetal growth through impaired placental function.

Dietary supplementation with 50 mg DHA/kg feed did not affect the numbers of total born or liveborn piglets. The average number of total born piglets was 19.8 and 19.7 in the control and DHA groups, respectively, which is comparable to the findings of McDermott, et al. [21], who reported 14.9 total born piglets in both control and fish oil-supplemented sows. In contrast, Smits, et al. [12] observed an increase in total born piglets from 9.7 to 10.7 when 3 g omega-3 PUFA/kg diet was supplied during late gestation and lactation. Adeleye, et al. [4] likewise demonstrated that dietary DHA supplementation reduced the number of stillborn piglets without affecting the total number born. In the current study, only 20% of the lowest DHA dosage used by Adeleye, et al. [4] was included, which may explain why no effects were seen on either litter size or the number of stillborn piglets. Most embryonic and fetal losses occur in early gestation [19], yet even supplementation with 4.3 g DHA/kg feed during the first 43 days of gestation did not affect total piglets born in hyperprolific sows (19.8 vs. 19.9 piglets) [39]. It may therefore be speculated that litter size in modern hyperprolific sow genotypes is already close to its biological limit. Consequently, although omega-3 fatty acids may improve aspects of reproductive physiology, their effects on litter size may be more apparent in less prolific genetic lines or in herds with lower baseline reproductive performance.

Individual piglet birth weight was not affected by DHA supplementation, which agrees with several previous studies [4, 14, 16, 40]. One study, however, reported reduced birth weight in piglets in sows fed salmon oil [21]. In this study, the proportion of piglets with high birth weight was also lower in sows fed DHA, though the mechanism behind this is unclear [21]. Additionally, no effect was observed on the within-litter variation in birth weight, which is consistent with findings from two other studies, where the variation was 214-216 g and 301-302 g, respectively [40]. Overall, increasing sow dietary omega-3 fatty acid intake appears to have limited effects on piglet birth weight. Although transfer of omega-3 fatty acids to the fetus may provide developmental benefits, these benefits do not necessarily translate into greater birth weight. It should be noted that neither tissue omega-3 concentrations nor measures of piglet vitality were assessed in the present study.

4.1. Colostrum Quality

Colostrum quality is critical for neonatal immune development, thermoregulation, and survival. Although the level of DHA supplementation was modest, colostrum from DHA-supplemented sows tended to contain higher DHA concentrations, indicating efficient transfer of dietary DHA into colostrum. Similar findings have been reported in several previous studies [6, 14, 16, 34]. Generally, the fatty acid profile in milk fat mirrors the fatty acid composition of the dietary fat [6, 41]. Since the sow diets in this study differed only in the level of DHA, there was no observed effect on colostrum concentrations of protein, fat, or lactose.

Consistent with several previous reports, dietary omega-3 fatty acid supplementation did not affect colostral immunoglobulin concentrations [14, 16, 42]. One study did find increased concentrations of immunoglobulin G in colostrum with a dietary n-6:n-3 ratio of 9:1 [40], which was similar to the dietary ratio in both groups of the current study. Chen, et al. [43] also observed increased concentrations of IgG, IgM, and IgA in colostrum when sows received 68 g n-3 PUFA/kg feed. However, Eastwood, et al. [14] evaluated similar dietary n-6:n-3 ratios using substantially lower levels of n-3 PUFA supplementation and found no effects on colostral IgA or IgG concentrations. Taken together, these findings suggest that colostral immunoglobulin concentrations may respond more strongly to higher dietary inclusions of n-3 fatty acids than to modest supplementation with DHA alone.

5. Conclusion

In conclusion, supplementation of sow diets with 50 mg DHA/kg feed throughout the entire reproductive cycle increased plasma glucose concentrations during late gestation and reduced the proportion of piglets weighing more than 1,600 g at birth. However, DHA supplementation did not affect litter size, average piglet birth weight, colostrum composition, or plasma cytokine concentrations. Parity influenced both reproductive and inflammatory parameters. Parity-3 sows had greater litter birth weights than parity-6 sows and exhibited lower plasma IL-6 concentrations and higher IL-10 concentrations than parity-2 sows. Furthermore, parity-6 sows had a higher proportion of piglets weighing less than 1,000 g at birth, whereas parity-3 and parity-4 sows had a higher proportion of piglets weighing more than 1,800 g compared with the other parity groups. Overall, long-term supplementation with a low level of DHA had limited effects on sow inflammatory status, colostrum quality, and piglet birth outcomes.

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Suppl. Table 1. Analyses of the diet composition of the ten diets used during the experimental period, and analyses of the fatty acid composition of the same ten diets. The ten diets consist of five control and five DHA diets.

Diet[1]	**Diet 1**		**Diet 2**		**Diet 3**		**Diet 4**		**Diet 5**	
Analyzed composition[2]**, g/kg**	**Control**	**DHA**	**Control**	**DHA**	**Control**	**DHA**	**Control**	**DHA**	**Control**	**DHA**
Analyzed samples, n	3	3	3	3	4	4	4	4	4	4
Dry matter	863	862	861	860	862	862	863	862	862	862
Crude protein	128	128	101	108	131	132	146	147	149	152
Crude fat	40	37	30	29	40	41	44	44	45	44
Crude ash	37	48	47	47	48	48	51	54	51	49
Lysine	7.79	8.03	4.87	4.93	6.38	6.13	9.3	9.35	9.87	9.68
Methionine	2.58	2.4	1.66	1.62	1.94	1.87	2.64	2.69	2.68	2.72
Cysteine	2.37	2.43	2.21	2.15	2.4	2.39	2.58	2.59	2.66	2.69
Threonine	5.49	5.25	4.04	3.95	4.85	4.68	6.28	6.30	6.30	6.34

Note: [1]Diet 1: Used during service. Diet 2: Day 0 to day 84 of gestation. Diet 3: Day 85 to day 110 of gestation. Diet 4: Day 111 of gestation to day 3 of lactation. Diet 5: Day 4 of lactation to weaning.
[2]All feed samples were analyzed in duplicate at Eurofins Steins Laboratories A/S. The diets do show some variations between the two groups within the same dietary period; however, these variations were presumed to be insignificant for the given experiment.

Suppl. Table 2. Fatty acid composition of control and DHA diets.

Diet[1]	**Diet 1**		**Diet 2**		**Diet 3**		**Diet 4**		**Diet 5**	
Analyzed composition [2]**, mg/kg**	**Control**	**DHA**	**Control**	**DHA**	**Control**	**DHA**	**Control**	**DHA**	**Control**	**DHA**
Analyzed samples, n (duplicates)	3	3	3	3	4	4	4	4	4	4
Myristic (C14:0)	40	75	60	87	68	149	60	108	59	105
Palmitic (C16:0)	4,698	4,523	4,369	3,826	5,574	5,478	5,307	4,961	5,377	5,315
Stearic (C18:0)	650	552	407	332	874	862	945	863	1,001	999
Oleic (C18:1 n-9)	4,531	3,886	2,657	2,113	5,524	5,301	6,177	5,520	6,390	6,533
Vaccenic (C18:1 n-7)	327	288	196	161	399	387	447	403	428	446
Linoleic (C18:2 n-6; LA)	14,632	13,241	10,107	8,503	16,914	16,306	17,825	16,312	17,003	17,125
α-Linolenic (C18:3 n-3; ALA)	1,598	1,441	1,039	861	1,945	1,858	1,962	1,843	1,948	1,971
Arachidic (C20:0)	69	59	43	35	96	96	103	94	106	108
Gondoic (C20:1 n-9)	91	93	81	70	103	101	92	94	93	92
Eicosadienoic (C20:2 n-6)	13	12	12	10	13	12	14	13	12	12
Eicosatrienoic (C20:3 n-3)	16	15	14	11	20	17	22	19	18	19
Behenic (C22:0)	90	75	60	50	105	108	124	112	116	122
Erucic (C22:1 n-9)	20	22	25	24	25	25	19	22	22	20
Docosahexaenoic (C22:6 n-3; DHA)[3]	3	63	3	29	1	68	0	46	2	32
Lignoceric (C24:0)	48	40	36	32	54	52	60	52	58	59
Total n-6 fatty acids	14,646	13,253	10,119	8,513	16,927	16,318	17,838	16,325	17,015	17,137
Total n-3 fatty acids	1,617	1,519	1,057	901	1,966	1,943	1,984	1,908	1,967	2,023
Ratio, n-6:n-3	9.1	8.7	9.6	9.5	8.6	8.4	9	8.6	8.7	8.5

Note: [1]Diets: Diet 1: Used during service. Diet 2: Day 0 to day 84 of gestation. Diet 3: Day 85 to day 110 of gestation. Diet 4: Day 111 of gestation to day 3 of lactation. Diet 5: Day 4 of lactation to weaning.
[2]All fatty acid analyses were performed in duplicate at the Department of Animal and Veterinary Sciences, Aarhus University.
[3]DHA were added as a supplement to the DHA group. It was added through DHAgold™, which has a declared content of C22:6 n-3 >17%, CP = 17%, crude fat = 56%, ash = 8.8%, crude fiber = 4.5%, carbohydrate = 12.4% (dsm-firmenich Animal Nutrition & Health, Basel, Switzerland).