



Effects of maternal supplementation of fish oil during late gestation and lactation on growth performance, fecal microbiota structure and post-weaning diarrhoea of offspring piglets

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Abstract

Homeostasis of gut microbiota is a critical contributor to growth and health in weaned piglets. Fish oil is widely reported to benefit health of mammals including preventing intestinal dysfunction, yet its protective effect during suckling-to-weaning transition in piglets remains undetermined. Low (30 g/d) and high (60 g/d) doses of n-3-rich fish oil were supplemented in sows from late gestation to lactation. Serum indicators and gut microbiota were determined to evaluate the effects of maternal fish oil on growth performance, immunity and diarrhea of piglets. DHA and EPA in the colostrum as well as serum of suckling and 1-week post-wean piglets were significantly and linearly increased by maternal supplementation of fish oil ($P < 0.05$). IGF1 and T3 in nursing and weaned piglets were significantly elevated by maternal fish oil ($P < 0.05$), and the increase of IGF1 was concerning the dosage of fish oil. Colostrum IgG, plasma IgG, IgM in suckling piglets, IgG, IgM and IgA in weaned piglets were significantly increase as maternal replenishment of fish oil increased ($P < 0.05$). Additionally, cortisol was significantly reduced in weaned pigs ($P < 0.05$), regardless of dosage. 16S rRNA sequencing revealed that α -diversity of fecal microbiota in nursery piglets, and fecal Lactobacillus genus, positively correlated with post-weaning IgA, was significantly increased by high dosage. Collectively, maternal fish oil during late pregnancy and lactation significantly promoted growth, enhanced immunity, and reduced post-weaning diarrhea in piglets, therefore facilitated suckling-to-weaning transition in piglets, which may be partially due to the altered gut microbial community.

Key words: Fish oil: Sow: Piglets: Weaning: Gut microbiota

Early weaning in infant mammals usually co-occurs with severe stress due to nutritional, physiological, environmental, social and psychological transitions. In the pig industry, piglets undergoing early weaning are often characterised by reduced growth and increased prevalence of diarrhoea after weaning; the latter is commonly considered as major health issue, as it always leads to substantial morbidity and mortality in piglets and results in huge economic losses in pig production⁽¹⁾. Although antibiotics and zinc oxide can effectively treat diarrhoea during the weaning process, the shortcomings of these measures also give rise to concerns regarding public health and environmental pollution⁽²⁾. Thus, there is an urgent need to identify effective alternatives to reduce or alleviate weaning-associated syndromes.

Intestinal microbiome undergoes dramatic changes from birth to weaning in piglets⁽³⁾, and accumulated data highlight the contribution of gut microbiota dysbiosis as a leading cause of post-weaning growth retardation or diarrhoea⁽⁴⁾. Meanwhile, the diet and environmental stimuli in early life exert

a significant intervention on the development of the gut microbiota in piglets. Direct evidence suggests that maternal administration of soluble fibre⁽⁵⁾ and resveratrol⁽⁶⁾ during pregnancy and/or lactation can improve the resistance to diarrhoea or diseases via reshaping the composition of the intestinal or fecal microbiota, while heat stress during late gestation can alter offspring's gut microbial colonisation⁽⁷⁾. Herein, it is a promising strategy to prevent or alleviate weaning stress and post-weaning diarrhoea by optimising maternal nutrition during gestation and/or lactation.

ω -3 PUFA consist of EPA, DHA, α -linolenic acid as well as DPA, an intermediate between EPA and DHA. Distinct from short- or medium-chain fatty acids which can serve as direct energy sources, essential ω -3 PUFA are always incorporated into cell membranes and thereby exert biological functions in relation to health and immunity⁽¹⁾. Recent reports also indicate that ω -3 PUFA also participate in the maintenance of gut homeostasis, which is associated with gut microbiota and intestinal health⁽⁸⁾.

Abbreviations: DAO, diamine oxidase; LPS, lipopolysaccharide.

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Furthermore, maternal ω -3 PUFA have been illustrated to transfer to offsprings via milk and provide multifarious benefits to the offspring, including better cognitive performance⁽⁹⁾, alleviation of inflammation⁽¹⁰⁾ or enhanced immunity^(11,12) by remodelling the gut microbiota community. The emerging effects of maternal fish oil on the growth performance, immune activity of nursery piglets^(13,14) encourage us to further verify whether ω -3 PUFA can effectively alleviate post-weaning diarrhoea and improve gut health in piglets.

In present study, ω -3 PUFA-rich fish oil was administrated from late gestation to lactation, and growth performance, immune activity, post-weaning diarrhoea and gut microbiota community in piglets were measured. The results are relevant to seeking potential strategies for improving the adaptation of piglets during the suckling-to-weaning transition.

Materials and methods

Animal management and sampling procedures were approved by the Animal Care and Use Committee of Northwest A&F University and were in accordance with the National Research Council's Guideline for the Care and use of Laboratory Animals.

Experimental scheme and animal care

This experiment was carried out at the Hengzhuang pig farm. A total of forty-five Landrace \times Yorkshire gestating sows (85 d of gestation, parity = 1, bodyweight 212.35 ± 1.61 kg, mean \pm SE) were randomly allocated to one of three dietary treatments according to their body weight and backfat thickness, with fifteen replicates per group. The dietary treatment comprised a basal commercial gestation or lactation diet (control, CON group; as listed in Supplementary Table S1) or the same basal diet supplemented with 30 or 60 g/d fish oil per sow from day 90 of gestation to weaning at day 21 of lactation, respectively, after 5 d of a pre-feeding period. Fish oil were offered to sows individually before feed was supplied in the morning, and sows generally finished the aliquots of fish oil within 5 min. Fish oil was obtained from Bailushi BioTech Ltd. Company. The concentrations of EPA and DHA were determined by GC-MS with the help of Panomix, and results shown that fish oil was comprised of 13.33% DHA and 5.40% EPA, and the ratio of EPA to DHA was 1:2.47.

Sows were housed in individual farrowing stalls (2.1 m \times 0.65 m) during the gestational period from day 85 to day 110. From day 100 of gestation to ab lactation, the sows were transferred into 2.2 m \times 1.8 m concrete-floored delivery pens. The day of parturition was defined as day 0 of lactation and the piglets were weaned at day 21 of lactation. Sows were fed twice a day (08:00 am and 14:00 pm) from late gestation to the first week of lactation and were fed *ad libitum* throughout lactation. According to the number of effective teats of sows, litters were standardised to \sim 12 piglets per sow within 24 h after birth by cross-fostering within treatments. Piglets were weighed individually on the 1st and 21th day of age. Nursing piglets got access to creep feed from 7 d after birth to the end of experiment (28 d after birth, or 1 week post-weaning), and the ingredients of creep feed were listed in Supplementary Table S2. All feeds in this study were provided by Shaanxi Power-Feed Technology Co., Ltd. The sows

and piglets had free access to water and were immunised according to the company's routine procedures.

Colostrum analysis

Within 2 h of the birth of the first piglet, eight sows per treatment were randomly selected for colostrum collection. Colostrum composition (milk fat, protein, lactose and casein, *et al.*) was determined via milk component analyzer (Foss FT 120), according to the manufacture's instruction. Targeted fatty acid quantitation was defined with GC-MS by Novogene. Briefly, milk fatty acids were solvent-extracted using 1% H₂SO₄ in methanol for 30 min at 80°C. The resulting methyl esters were extracted by N-hexane. FA methyl esters were separated and quantified using Thermo Trace and ISQ7000 GC-MS system equipped with a Thermo TG-FA methyl ester capillary column (50 m \times 0.25 mm ID \times 0.20 μ m). The sample injection volume was 1 μ l with a split ratio of 8:1. The temperature program was as follows: initial temperature 80°C for 1 min, increased by 20°C/min to final temperature 160°C and held for a further 1.5 min, increased by 3°C/min to final temperature 196°C and held for a further 8.5 min, increased by 20°C/min to final temperature 250°C and held for a further 3 min. The temperature of the interface was 250°C. Identification of fatty acid peaks was made by comparing their retention times to that of known FA methyl ester standards (Sigma-Aldrich). A 5-fold titration of mixture (containing fifty-one fatty acid methyl esters, 4000 μ g/ml) was used to generate a standard curve. Individual fatty acid peaks were quantified as absolute values (mg/ml). Fatty acid composition in the serum of piglets was determined in a similar procedure as described above.

Blood analysis

On days 21 and 28, one piglet (close to the average body weight of litter) delivered from the sows (the same as those for foremilk sampling) was selected for blood sampling from the anterior vena cava. Serum biochemical indicators were analysed with a Beckman Coulter AU5800 biochemical analyzer (Beckman Coulter, Inc.). Serum growth factors, cortisol, immunoglobulins and intestinal endotoxins were determined by commercial kits (PoFAine IGF1, T3, T4, insulin, cortisol, IgA, IgG, IgM, zonulin, diamine oxidase and D-lactate ELISA kits) from Shanghai Hengyuan Biological.

Fecal microbial analysis

Fresh feces were collected directly by messaging the rectum of the corresponding piglets at day 21 of age. Stool samples were cooled on dry ice and stored at -80°C until 16S rRNA sequencing by Biomarker Technologies. Microbial DNA was extracted with DNeasy PowerSoil kit as the protocol. The V3–V4 hyper variable regions within 16S rRNA were augmented with universal primers (338F-806R), and the amplicons were screened on Illumina Novaseq platform (Illumina) under Illumina's standard procedure. The bioinformatic analysis was completed at the Biomarker biocloud platform. Briefly, paired-end reads were merged from the original DNA fragments by FLASH (version 1.2.7), which were further filtered by UCHIME. The clean reads

Table 1. Effects of maternal fish oil on colostrum composition

	Fish oil, % of diet						P-value
	0		30 g/d		60 g/d		
	Mean	SEM	Mean	SEM	Mean	SEM	
Milk fat, %	4.46	0.58	4.55	0.06	4.59	0.12	0.553
Protein, %	13.37	0.24	13.63	0.12	13.48	0.18	0.618
Non-fat solids, %	18.68	0.19	18.25	0.19	18.51	0.31	0.464
Total Solids, %	24.70	1.75	24.32	0.14	23.46	0.84	0.329
Lactose, %	3.23	0.12	3.39	0.16	3.55	0.10	0.263
Casein, %	9.24	0.21	9.58	0.17	9.48	0.11	0.389
Density, SG	1027.75	0.48	1026.75	0.75	1028.50	0.87	0.274
Urea nitrogen, mg/100 l	666.70	29.63	623.10	24.39	647.55	25.27	0.531

Data were presented as mean \pm SEM, *n* 8.

were clustered into operational taxonomic units by USEARCH (version 10.0) on the basis of 97% sequence similarity. All augmented tags were glossed and allied against SILVA database (version 132) through Ribosomal Database Project classifier (version 2.2, 80% confidence threshold).

Diarrhoea index

Feces consistency (from days 22 to 28 post-weaning) was scored using a subjective score on a 3-point scale: 0, normal, well-formed and solid feces; 1, soft and formed feces; 2, sloppy, loose and semiliquid feces; 3, diarrhoea, watery and unformed feces. Piglets were considered to be diarrheic when the diarrhoea score was 2 or above⁽¹⁵⁾. Diarrhoea rate (%) = Σ (the number of piglets/pen \times days of diarrhoea)/(total number of trial piglets \times 7 d). Diarrhoea index = the sum of diarrhoea scores/pen/(total number of trial piglets \times 7 d).

Statistical analysis

Microbiota diversity was assessed based on the number of OUT and Shannon-index using the *phyloseq* package of R⁽¹⁶⁾, and differences in bacterial communities among diet groups were evaluated with permutational multivariate analysis of variance (PERMANOVA) using UniFrac distance matrices based on both unweighted and weighted UniFrac distances using QIIME 2 (2019.4)⁽¹⁷⁾. All other data were analysed with one-way ANOVA procedure (SPSS 20.0 software for Windows, SPSS Inc.), statistical significance was declared at $P < 0.05$ and a trend towards significance was considered at $0.05 < P < 0.10$. *Post-hoc* mean separation with a Tukey adjustment was performed when the ANOVA resulted in a P -value < 0.05 . Orthogonal polynomial contrasts were conducted to determine linear (L) and quadratic (Q) effects of supplemental fish oil. The interactions between the abundance of fecal microbiota and serum parameters were analysed by Spearman's correlation.

Results

Maternal administration of fish oil altered fatty acid composition in colostrum and the blood of piglets

Dietary administration of fish oil from late gestation to lactation did not affect feed intake, body weight and backfat loss of the

dams throughout the experiment (as listed in Supplementary Table S3), and the numbers of piglets born, born alive, healthy or weak or stillborn newborns, litter weight alive at parturition and average weight of piglets born alive were comparable among different groups (online Supplementary Table S4). Also, the addition of fish oil during late gestation and lactation did not significantly change the composition of colostrum regarding milk fat, protein, lactose or casein contents (Table 1). For fatty acid composition in colostrum, dietary supplementation of fish oil significantly heightened the concentrations of EPA, DHA and ω -3 PUFA in the colostrum with 3–5-fold, 5–8-fold and \sim 2-fold increase, respectively, and led to significantly reduced ratio of ω -6/ ω -3 PUFA as maternal intake of fish oil increased, while the content of ω -6 PUFA was not greatly altered (Table 2). Meanwhile, α -linolenic acid, another ω -3 PUFA, remained unaltered by maternal fish oil administration. In line with the changes in colostrum, plasma EPA and DHA in suckling piglets were significantly elevated by maternal exposure to fish oil in a dose-dependent way, and the ω -6/ ω -3 PUFA ratio was dramatically decreased, especially in the lower (30 g/d) treatment group (Table 2).

Maternal administration of fish oil promoted the growth of piglets

To balance the carrying ability of each sow, newborns were cross-fostered within the same group, and thus the born litter size and weight were comparable among groups. Twenty-one days later, litter size at weaning was nearly the same, while the weaning weights of litters tended to be elevated by maternal intake of fish oil, reaching a significant level in the high dose group. The difference in litter weight gain was not significant owing to the higher variance within groups (Table 3).

Serum IGF1 levels in suckling piglets fostered by sows treated with fish oil were significantly higher than those in the control group. Moreover, serum T3 was also significantly escalated by maternal supplementation of 30 g/d fish oil, whereas T4, insulin and cortisol remained unchanged (Table 4). The effects of maternal fish oil treatment on serum growth factors lasted for 1 week post-weaning, as significant increase was also observed in serum IGF-1, especially in the 60 g/d treatment group, and circulating T3 instead of T4 levels were significantly increased by the maternal intake of fish oil (Table 4). Notably, the

Table 2. Effects of maternal fish oil on fatty acid composition in colostrum and blood of sucking piglets ($\mu\text{g/ml}$)

		Treatment			RMSE(Root Mean Square Error)	P-value		
		0	30 g/d	60 g/d		T	L	Q
Colostrum	EPA	25.24 ^b	99.633 ^a	69.32 ^{a,b}	37.41	0.018	0.030	0.002
	DHA	42.01 ^c	207.37 ^b	313.68 ^a	136.90	0.001	0.005	0.001
	ALA	318.58	315.35	375.72	33.96	0.583	0.960	0.309
	ω -3 PUFA	385.83 ^b	622.35 ^{a,b}	758.72 ^a	188.67	0.017	0.110	0.009
	ω -6 PUFA	3244.98	3565.40	3526.68	174.89	0.818	0.569	0.802
	ω -6 PUFA/ ω -3 PUFA	8.54 ^a	5.98 ^b	4.67 ^b	1.97	0.001	0.013	0.001
Suckling piglets	EPA	3.21 ^b	10.81 ^a	11.28 ^b	4.53	0.005	0.175	0.143
	DHA	21.95 ^b	57.82 ^a	40.87 ^a	17.94	0.004	0.441	0.060
	ALA	6.03	8.50	6.80	1.26	0.533	0.731	0.297
	ω -3 PUFA	31.19	64.34	44.79	16.66	0.864	0.399	0.079
	ω -6 PUFA	278.00	338.43	241.18	49.10	0.455	0.634	0.255
	ω -6 PUFA/ ω -3 PUFA	8.87 ^a	5.42 ^b	6.00 ^{a,b}	1.85	0.050	0.050	0.098

Values are medians unless otherwise indicated. ALA, α -linolenic acid.

^{a-b}Values in rows with different superscript letters differ significantly ($P < 0.05$, $n = 8$).

Table 3. Effects of maternal administration of fish oil during late-pregnancy and lactation on growth of piglets

	Treatment			RMSE	P-value		
	0	30 g/d	60 g/d		T	L	Q
Litter size after cross-foster, n	11.8	12.00	12.27	4.09	0.682	0.386	0.943
Litter weight after cross-foster, kg	15.28	16.08	16.13	3.55	0.639	0.402	0.667
Litter size at weaning, n	10.67	11.00	11.13	1.91	0.453	0.224	0.762
Litter weight at weaning, kg	73.72 ^b	80.53 ^{a,b}	81.58 ^a	15.80	0.110	0.048	0.476
Litter weight gain, kg	58.44	63.98	65.45	13.54	0.129	0.056	0.515

Values are medians unless otherwise indicated.

^{a-b}Values in rows with different superscript letters differ significantly ($P < 0.05$, $n = 15$).

Table 4. Effects of maternal administration of fish oil during late-pregnancy and lactation on serum growth factors in piglets

		Treatment			RMSE	P-value		
		0	30 g/d	60 g/d		T	L	Q
Day 21 of age	IGF1, $\mu\text{g/ml}$	8.74 ^b	9.63 ^a	10.08 ^a	0.84	0.004	0.001	0.468
	T3, pmol/l	82.45 ^b	100.56 ^a	89.38 ^{a,b}	11.04	0.003	0.140	0.001
	T4, pmol/l	377.54	367.82	362.78	78.10	0.943	0.740	0.952
	Insulin, mmg/l	50.84	47.84	48.41	4.58	0.452	0.340	0.415
Day 28 of age	IGF1, $\mu\text{g/l}$	14.14 ^b	14.92 ^{a,b}	15.84 ^a	1.08	0.005	0.001	0.863
	T3, pmol/l	140.35 ^b	154.13 ^a	152.40 ^a	10.53	0.019	0.021	0.076
	T4, pmol/l	627.01	645.47	640.92	48.19	0.779	0.626	0.616
	Insulin, mmg/l	59.55	57.25	58.65	3.38	0.463	0.628	0.258

Values are medians unless otherwise indicated.

^{a-b}Values in rows with different superscript letters differ significantly ($P < 0.05$, $n = 8$).

responses of plasma IGF1 in offsprings to maternal fish oil were strictly dependent on dosages, more intake of fish oil, much higher IGF1 levels.

Circulating cortisol, a well-known stress hormone, was not affected ($P = 0.114$) by the maternal addition of fish oil in nursing piglets but was significantly reduced by a third in weaned piglets (Fig. 1).

The concentration of IgG in colostrum tended to be increased by the maternal addition of fish oil, and a significant increase was observed in the high dose group (60 g/d, Table 5), while IgM ($P = 0.882$) and IgA ($P = 0.150$) in colostrum were not affected by maternal supplementation of fish oil. In the serum of suckling piglets (21 d of age), IgG and IgM were significantly elevated by

maternal fish oil supplementation in a dose-dependent manner (Table 5), leaving IgA unchanged. However, 1 week post-wean, all of plasma IgG, IgM and IgA levels were significantly elevated by maternal supplementation with fish oil, and more addition of fish oil, more immunoglobins.

Maternal supplementation of fish oil improved gut health of piglets

Zonulin and D-lactate in the blood of suckling piglets were significantly decreased by maternal treatment with fish oil, reaching a significant level with maternal supplementation of 60 g/d fish oil. Serum diamine oxidase (DAO) in the nursery

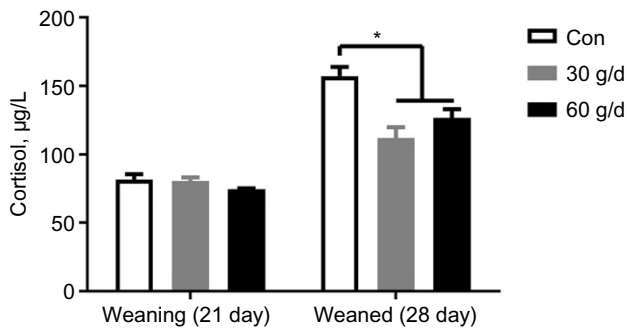


Fig. 1. Effects of maternal administration of fish oil during late pregnancy and lactation on serum concentration of cortisol in piglets. *Means significantly differ between groups ($P < 0.05$, $n = 8$).

piglets was also significantly reduced by maternal supplementation of fish oil, and a linear response of DAO to maternal intake of fish oil was observed. However, serum lipopolysaccharide (LPS) in suckling piglets was not largely affected by maternal administration of fish oil. Notably, 1 week post-wean (28 d of age), serum zonulin, DAO, D-lactate and LPS were significantly lower in fish oil offspring than those of the control; this was in accordance with the lower incidence of diarrhoea in piglets raised by fish oil sows compared with sows in the control group (Table 6). Neither serum C-reactive protein in nursing ($P = 0.166$) nor post-weaned piglets ($P = 0.711$) were affected by maternal fish oil supplementation.

As shown in Fig. 2(a), the Abundance-based Coverage Estimator (ACE), Chao1, Simpson and Shannon indices of fecal microbiota were similar among groups, and the PD_{whole} tree index was significantly increased by the maternal treatment with fish oil, suggesting that the α -diversity was largely increased by maternal supplementation with fish oil.

As shown in Fig. 2(b), at the phylum level, the enrichment of *Chloroflexi* and *Acidobacteria* was significantly increased by the low dose (30 g/d) but not the high dose (60 g/d) of fish oil. Furthermore, family *Lactobacillaceae* was significantly reduced by the low but induced by the high dose, and *Acidaminococcaceae* was significantly repressed by maternal supplementation of fish oil, regardless of the dosage. At the genus level, the changes in *Lactobacillus* were similar to that of *Lactobacillaceae*. The abundance of the genus *uncultured_bacterium_f_Muribaculaceae* was significantly

increased by the high dose of maternal fish oil, while *Phascolarctobacterium* was significantly decreased by maternal fish oil under both high and low doses (Fig. 2(c)).

Pearson correlation analysis revealed a moderate and significant interaction between fecal microbiota and serum factors in near-weaning piglets (Fig. 3 and online Supplementary Table S5). At the phylum level, the abundance of *Firmicutes* was positively correlated with serum IgG and negatively correlated with serum zonulin, while the ratio of *Bacteroidetes/Firmicutes* was positively correlated with serum zonulin. *Synergistetes* was negatively associated with circulating IgG levels. Fecal *Chloroflex* was positively correlated with serum IgM and T3 concentrations. At the family level, *Ruminococcaceae* was positively correlated with serum IgG. *Christensenellaceae* was negatively correlated with circulating zonulin. *Lachnospiraceae* was positively correlated with both serum IgG and T4. Fecal *Acidaminococcaceae* was positively correlated with circulating DAO. *Enterobacteriaceae* was negatively associated with serum IgM, while *Synergistaceae* was negatively correlated with serum IgG. Notably, the genus *uncultured_bacterium_f_Christensenellaceae* was negatively correlated with serum zonulin. *Phascolarctobacterium* was positively associated with circulating DAO, and *Escherichia-Shigella* was negatively correlated with serum IgM.

Moderate and significant interactions between fecal microbiota at weaning and post-weaning serum indicators were also observed (Fig. 4 and online Supplementary Table S6). At the phylum level, the abundances of *Firmicutes*, *Bacteroidetes* and the ratio of *Bacteroidetes/Firmicutes* were not significantly correlated with serum parameters in weaned piglets, although the phylum *Synergistetes* was negatively associated with serum IgA and IGF1 concentrations. *Actinobacteria* was positively correlated with circulating zonulin, and *Chloroflexi* and *Acidobacteria* were negatively associated with serum cortisol. At the family level, *Ruminococcaceae* was positively correlated with serum IgM, *Lactobacillaceae* was positively correlated with serum IgA; *Lachnospiraceae* was negatively associated with serum LPS; *Bacteroidaceae* was positively correlated with serum zonulin; *Acidaminococcaceae* was negatively correlated with serum T3 and positively with D-lactate; *Enterobacteriaceae* was positively correlated with serum C-reactive protein, and *Synergistaceae* was negatively correlated with IgA and IGF1.

Table 5. Effects of maternal administration of fish oil during late-pregnancy and lactation on serum immunoglobulins content in colostrum and blood of piglets

		Treatment			RMSE	P-value		
		0	30 g/d	60 g/d		T	L	Q
Colostrum	IgG, µg/ml	127.48 ^b	133.33 ^{a,b}	149.62 ^a	18.84	0.067	0.530	0.026
	IgM, µg/ml	17.69	17.99	17.69	1.24	0.882	0.669	0.803
	IgA, µg/ml	20.50	20.61	21.43	0.98	0.150	0.831	0.056
Suckling piglets (day 21 of age)	IgG, µg/ml	145.39 ^b	158.70 ^{a,b}	176.27 ^a	22.04	0.022	0.007	0.809
	IgM, µg/ml	9.72 ^b	17.68 ^a	16.48 ^a	4.87	0.001	0.002	0.011
	IgA, µg/ml	6.55	7.79	8.34	1.99	0.237	0.102	0.701
Weaned piglets (day 28 of age)	IgG, µg/ml	297.23 ^b	313.97 ^b	332.81 ^a	21.32	0.002	0.001	0.889
	IgM, µg/ml	10.30 ^b	13.24 ^{a,b}	15.85 ^a	1.03	0.027	0.008	0.921
	IgA, µg/ml	19.93 ^b	20.40 ^b	22.18 ^a	1.42	0.002	0.001	0.201

Values are medians unless otherwise indicated.

^{a-b}Values in rows with different superscript letters differ significantly ($P < 0.05$, $n = 8$).

Table 6. Effects of maternal administration of fish oil during late-pregnancy and lactation on serum endotoxins in piglets

		Treatment			RMSE	P-value		
		0	30 g/d	60 g/d		T	L	Q
Day 21 of age	Zonulin, /ml	470.14 ^a	460.02 ^a	423.28 ^b	36.16	0.029	0.012	0.371
	DAO, pg/ml	224.55 ^a	168.25 ^b	130.96 ^b	50.97	0.002	0.002	0.554
	D-lactate, µg/l	1015.34 ^a	944.42 ^{a,b}	816.03 ^b	158.88	0.050	0.017	0.667
	LPS, pg/ml	207.52	226.78	200.84	40.51	0.485	0.093	1.416
	CRP, µg/l	2163.94	2034.87	2139.86	135.09	0.166	0.731	0.065
Day 28 of age	Zonulin, /ml	545.94 ^a	513.87 ^{a,b}	484.82 ^b	42.54	0.018	0.005	0.929
	DAO, pg/ml	226.67 ^a	209.82 ^b	208.94 ^b	12.89	0.007	0.005	0.112
	D-lactate, µg/l	1311.55 ^a	1152.27 ^b	1102.58 ^b	137.23	0.005	0.002	0.287
	LPS, pg/ml	275.15 ^a	258.64 ^{a,b}	224.12 ^b	40.56	0.047	0.016	0.596
	CRP, µg/l	2205.92	2164.91	2186.92	89.03	0.711	0.704	0.469
	Diarrhoea rate, %	21.88	11.05	9.58	3.56	0.012	0.024	0.465
	Diarrhoea index	0.53	0.35	0.24	0.08	0.009	0.011	0.391

Values are medians unless otherwise indicated. DAO, diamine oxidase; LPS, lipopolysaccharide; CRP, C-reactive protein.
^{a-b}Values in rows with different superscript letters differ significantly ($P < 0.05$, $n = 8$).

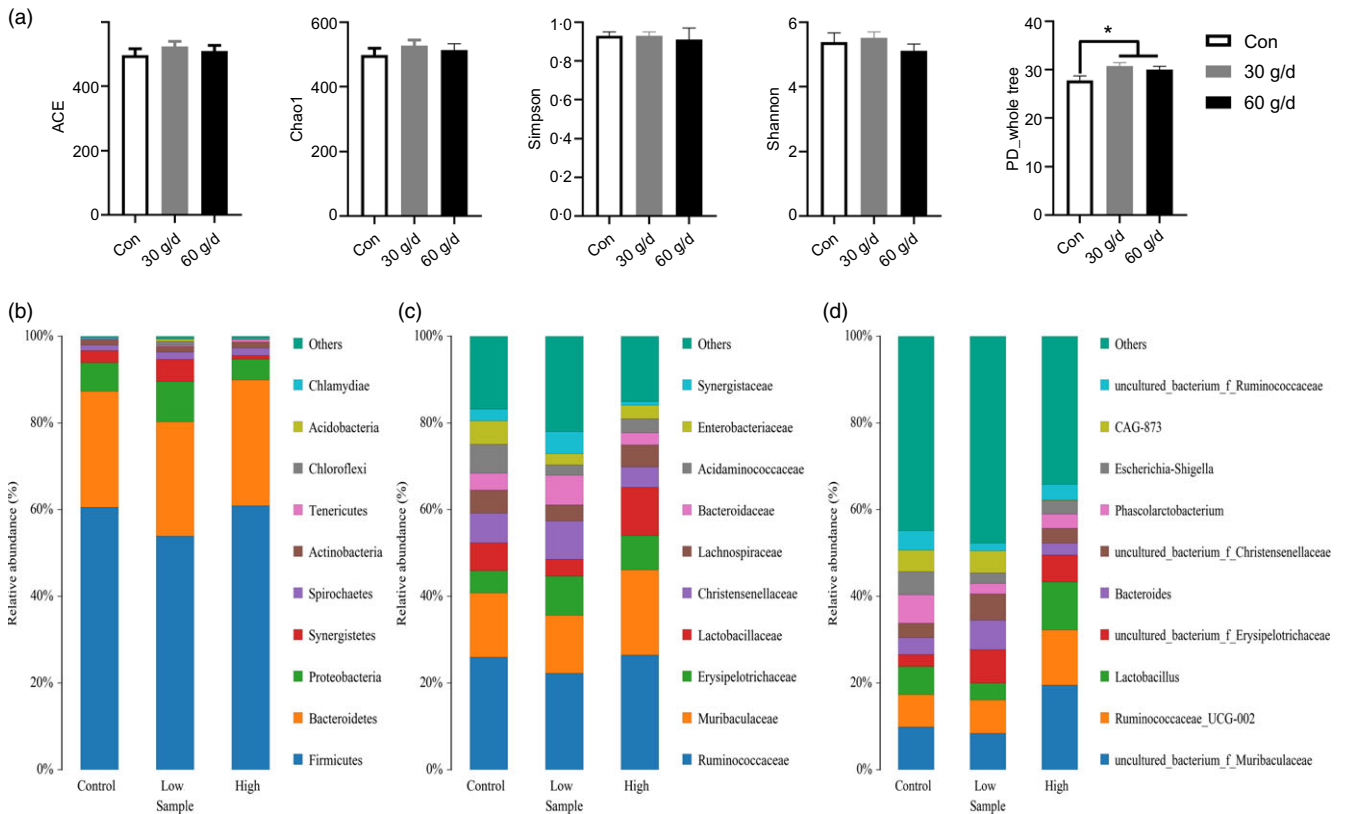


Fig. 2. Effects of maternal administration of fish oil during late-pregnancy and lactation on fecal microbiome in weaning piglets. *Means significantly differ between groups ($P < 0.05$, $n = 8$).

At the genus level, *uncultured_bacterium_f_Muribaculaceae* was negatively correlated with circulating insulin; *Ruminococcaceae_UCG-002* was strongly and positively correlated with IgG and T4 levels, and a positive interplay between *Lactobacillus* and serum IgA was observed. Moreover, *Bacteroides* was positively correlated with serum zonulin; *Phascolarctobacterium* was negatively associated with T3; *Escherichia-Shigella* was positively correlated with serum C-reactive protein, and *uncultured_bacterium_f_Ruminococcaceae* was positively interplayed with serum DAO.

Discussion

ω -3 PUFA, especially EPA and DHA, are essential fatty acids that can only be obtained from the diet, and insufficient intake of EPA or DHA can lead to a high risk of maternal under-nutrition and impaired fetal growth. Therefore an extra 200 mg/d DHA, as fatty acid from a sea fish once a week, is recommended for pregnant women⁽¹⁸⁾. Maternal replenishment of marine oil has been documented to improve reproductive performance in sows^(14,19) and reduce inflammation in weaned piglets⁽²⁰⁾, although the related mechanism remains unknown.

Correlation heatmap

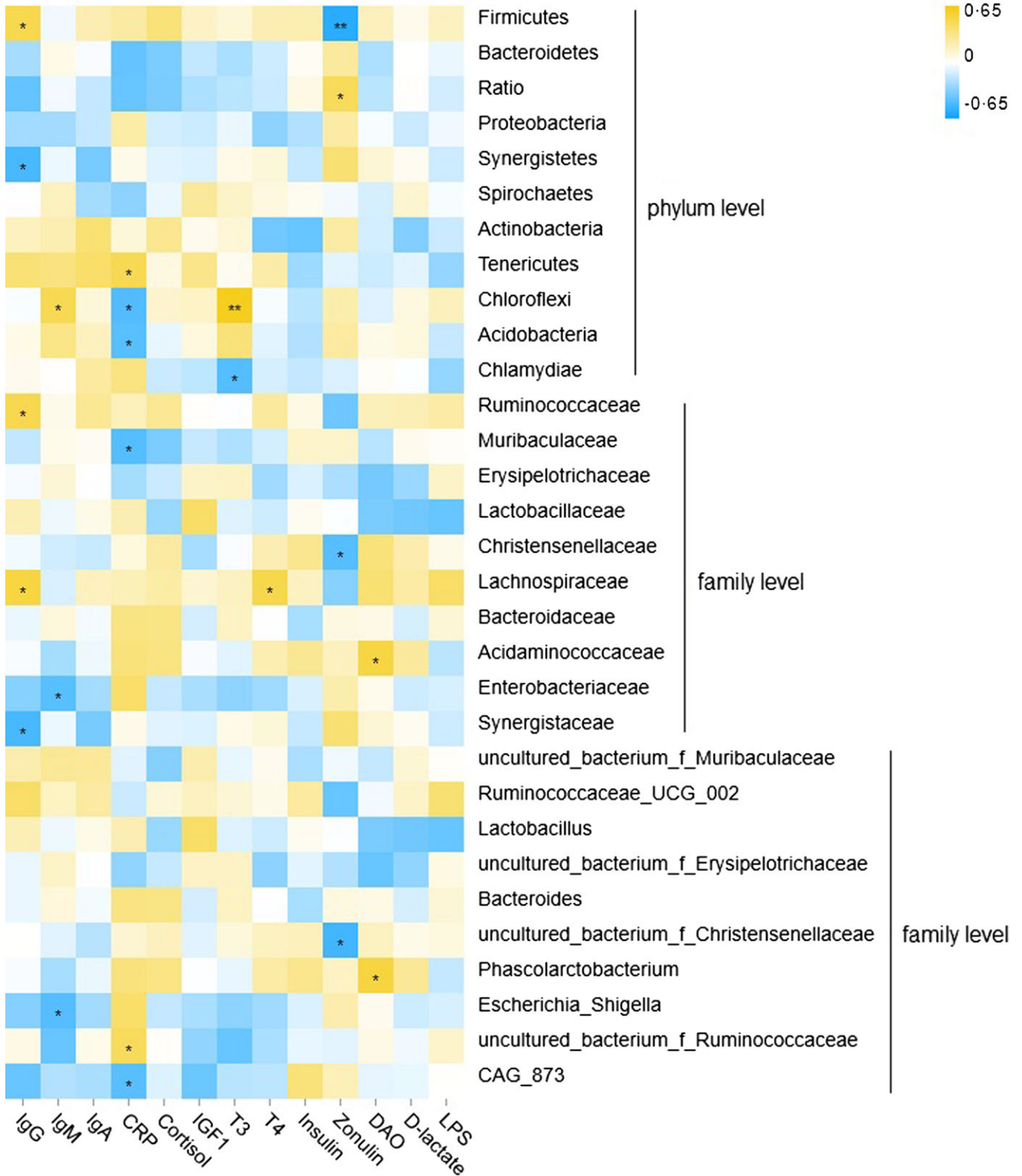


Fig. 3. Correlation analysis between fecal microbiota and serum growth factors in suckling piglets. * $P < 0.05$; ** $P < 0.01$.

Correlation heatmap

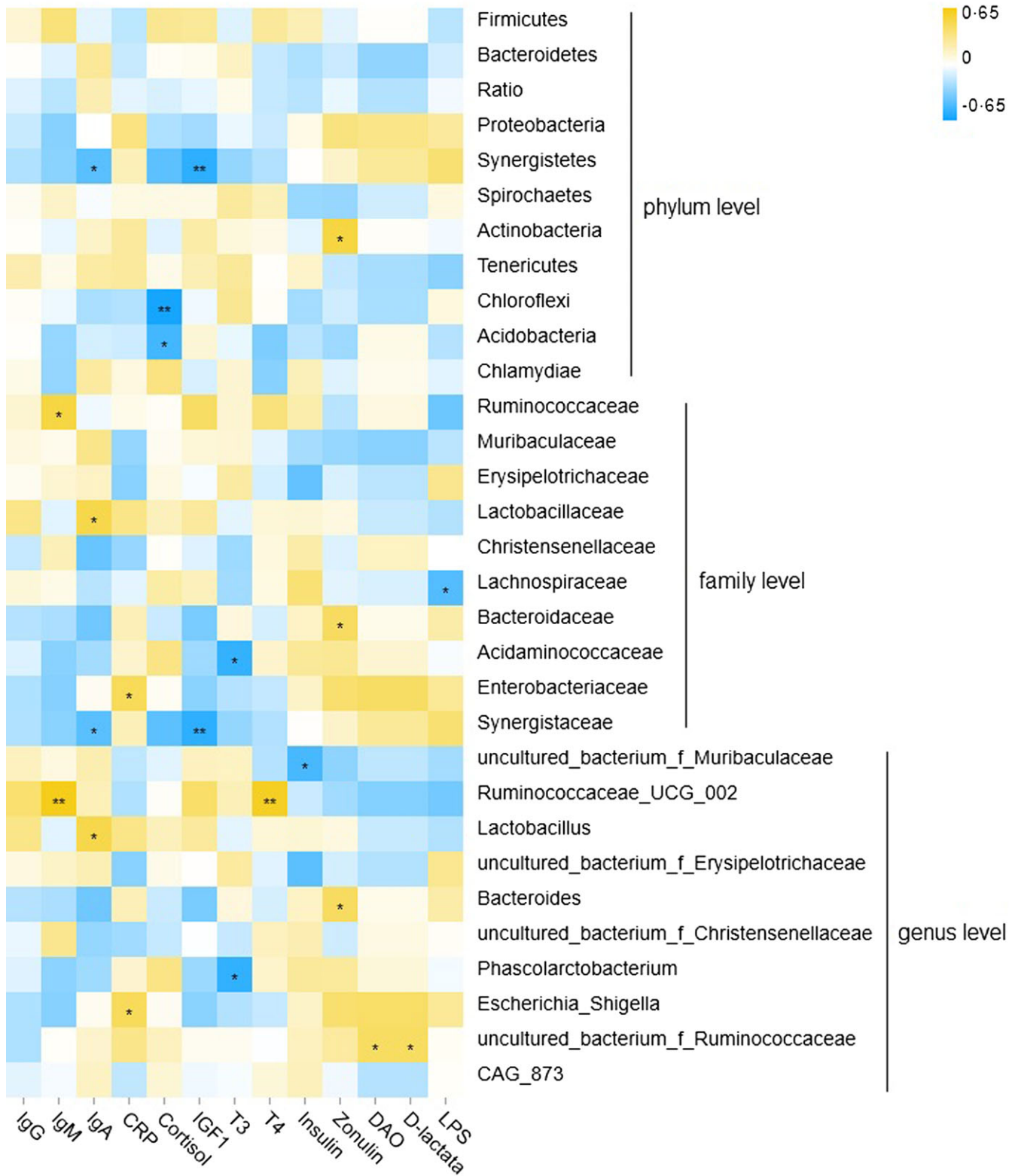


Fig. 4. Correlation analysis between fecal microbiota and serum growth factors in weaned piglets. * $P < 0.05$; ** $P < 0.01$.

In the present study, maternal intake of fish oil during late gestation and lactation led to elevated contents of EPA and DHA in colostrum and piglets' blood. Similarly, a double-blind, randomised, controlled trial in humans revealed that increased ω -3 PUFA in breast milk and infant DHA status was associated with supplementation during pregnancy⁽²¹⁾. Furthermore, dose-dependent increases in plasma levels of EPA and DHA in offspring have been reported in shorthair cats⁽²²⁾ and beagle dogs⁽²³⁾. Thus, maternal treatment of EPA and DHA could pass through to the next generation via colostrum and milk, elevating the intake of ω -3 PUFA in newborns.

Meanwhile, litter weight at weaning, especially in the high dose group, was tended to be increased upon exposure to fish oil in the prenatal and early postnatal life in this study, although the average birth weights were comparable, which was also observed in calves with a similar experiment design⁽¹²⁾. These data encouraged us to explore whether the endocrinal factors related to growth were altered by fish oil treatment, and results showed that exposure to fish oil in early life significantly escalated serum IGF1 and T3 in weaning and weaned piglets; furthermore, the increase of IGF1 and T3 by fish oil fits well in a linear model.

IGF1 is recognised as one of the predominant modulators during postnatal growth, whose expression was demonstrated to be associated with body height and loin muscle area in pigs⁽²⁴⁾. A previous study showed that long-term (16 weeks) supplementation of fish oil significantly increased plasma IGF1 in 13- to 15-year-old boys⁽²⁵⁾, and n -3 supplementation was documented to prevent the decrease of serum IGF1 induced by CVD in middle-aged men⁽²⁶⁾ or a low-fat diet in postmenopausal women⁽²⁷⁾. In addition, short-term (11 d) of EPA administration was reported to reverse the circulating IGF1 reduced by arthritis in rats⁽²⁸⁾. Considering that the liver is the major source of plasma IGF1 in postnatal piglets as a response to pituitary growth hormone plus⁽²⁹⁾, the detailed mechanism concerning how ω -3 PUFA modulate hepatic IGF1 transcription, translation and secretion deserves further investigation.

In addition to IGF1, plasma T3 levels in the piglets were also dramatically increased by the maternal intake of fish oil in our study, in accordance with a recent survey in humans showing that prenatal exposure to fish oil was positively associated with the T3/T4 ratio in cord blood of newborns⁽³⁰⁾. However, an earlier study showed that maternal intake of fish oil during lactation exerted no significant effects on serum T3 or T4 in male offspring of rats⁽³¹⁾. Moreover, in a randomised double-blind controlled trial on obese subjects, treatment of DHA and EPA (ratio = 1:1.5) for 4 weeks significantly increased T4 but not T3 in fasting blood⁽³²⁾. These conflicting results led us to suppose that the thyroid status might be a secondary consequence of fish oil treatment and that this was influenced by other factors, for example, the cross-talk between thyroid-stimulating hormone and IGF1 receptor⁽³³⁾.

Suckling-to-weaning transition always places piglets at high risk of severe stress thus impede growth. Notably, plasma concentrations of cortisol, stress hormone in pigs, were significantly reduced in weaned piglets nursed by sows fed with fish oil, compared with their counterparts in the control group, although the cortisol levels in weaning piglets were comparable,

in accordance with a previous study where maternal replenishment of fish oil protected weaned piglets from acute inflammation induced by LPS challenge⁽²⁰⁾. Thus, we suggest that the reduction of cortisol by fish oil might relieve harmful stress and facilitate the adaptation to weaning in piglets.

Fish oil has been long known to affect immunity. Unlike humans, IgG is the predominant immunoglobulin in porcine colostrum, which provides essential protection for the newborns, especially within the first 24 h postpartum when the gut is 'open'⁽³⁴⁾. Here, maternal supplementation of fish oil during late gestation and lactation tended to increase IgG in the colostrum, in line with a previous study where dietary administration of DHA plus EPA during late gestation and early lactation led to an increase in IgG(T) in mare's milk⁽³⁵⁾. Consistent with this, plasma IgG in suckling piglets was also elevated by exposure to fish oil in early life, a result observed in calves⁽¹²⁾ and foals⁽³⁵⁾ with EPA and DHA supplementation in uterine and/or early life. Also, the level of circulating IgM was increased in suckling piglets along with IgG and IgA in weaned piglets born from sows supplemented with fish oil, which is an attracting result for us. The effective transfer of immunoglobins ceases when 'gut closure' occurs near weaning⁽³⁴⁾. Thus, we hypothesised that the increased immunoglobins in weaned piglets, especially IgA, might be principally due to *de novo* synthesis.

IgA is the major immunoglobulin secreted by the parotid, nasal, bronchial and intestinal mucosa in piglets⁽³⁴⁾. Fish oil has been demonstrated to protect weaned piglets from LPS challenge via enhancing intestinal barrier function⁽³⁶⁾, and thus the increase of serum IgA in weaned piglets may be attributed to improve gut function. The gut barrier function of neonatal piglets is known to be relatively weak, gradually becoming strong as growing; exocrine secretion of three products from intestinal contents, zonulin, DAO and D-lactate, are commonly used as indicators of gut permeability⁽³⁷⁾. As expected, in our study, circulating zonulin, DAO and D-lactic acid in weaning and weaned piglets delivered from sows fed fish oil were significantly decreased compared with those of the control group. Moreover, plasma LPS, a typical endotoxin produced by pathogenic bacteria, was largely reduced by maternal fish oil supplementation, especially with the high-dose treatment in weaned piglets. Taken together, the results strongly suggested that maternal intake of fish oil was favourable to gut development and intestinal health of weaned piglets.

Gut health is tightly associated with the composition of the intestinal microflora; accumulating evidence has highlighted a substantial influence of dietary fish oil or ω -3 PUFA on the gut microbiome (see the latest review⁽⁸⁾). Moreover, the positive modulation of *in utero* and early life intake of ω -3 PUFA, particularly EPA and DHA, can extend to adolescence and adulthood⁽³⁸⁾. Thus, we moved on to explore the fecal microbiota structure in the weaning piglets.

Notably, the current data indicated that more or less exposure to maternal fish oil exerted different effects on the structure of the gut microbiota in the weaning piglets. For example, the family *Acidaminococcaceae* was dramatically reduced by maternal supplementation with fish oil and to a greater extent in the high-dosage treatment group, while the genus *Phascolarctobacterium* was reduced by the low dosage of fish



oil. Interestingly, the abundance of *Lactobacillus* in the family *Lactobacillaceae* responded to fish oil in a binary linear pattern, being significantly reduced by the lower yet increased by the higher dosage of maternal fish oil. A newly published report suggests that dietary ω -3 PUFA could be partially metabolised by anaerobic *Lactobacilli* in the distal intestine of pigs, thus directly influencing the gut microbiota distribution⁽¹⁾. As such, we assume that dietary ω -3 PUFA might not be entirely digested in the gut, as treatment with lower dosages of fish oil was not sufficient to promote or even repress the growth of *Lactobacillus* in present study.

The interplay between the gut microbiota and the host occurs in multiple and perplexing ways, and the functions and mechanic pathways of most microbiota species remain unconfirmed. A recent report showed that *Akkermansia muciniphila*, an emerging superstar against obesity and metabolic disorders, could secrete an 84 kDa protein, P9 and the latter could induce systemic secretion of glucagon-like peptide-1, an important factor modulating energy metabolism⁽³⁹⁾. Thus, we are curious whether gut microbiota impacts systemic immunity or serum growth-related factors in piglets.

Correlation analysis revealed an interaction between the gut microbiota and serum immunoglobulins. For example, *Lachnospiraceae* family was significantly and positively correlated with serum IgG in weaning piglets, which falls in line with an earlier study where *Lactobacillus* agents were shown to increase serum IgG in piglets⁽⁴⁰⁾. The abundance of *Escherichia coli-Shigella*, the enrichment of which is relevant to human dysbacteriosis⁽⁴¹⁾ and calf diarrhoea⁽⁴²⁾, was negatively correlated with serum IgM in weaning piglets.

Although the composition of the gut microbiome undergoes a dramatic change during the suckling-to-weaning transition⁽⁴³⁾, it was demonstrated that the gut microbiota at the time of weaning influenced the development of post-weaning diarrhoea⁽⁴⁴⁾. Here, *Lactobacillus* in weaning piglets was positively correlated with serum IgA in weaned piglets, and the abundance of *Ruminococcaceae_UCG_002*, a butyrate producer⁽⁴⁵⁾ in weaning piglets, was positively correlated with serum IgM and T4 in weaned piglets. A significant and negative association between growth factor, IGF1 in weaned pigs, and the family *Synergistaceae* were also observed. It is noteworthy that the tendency of a negative correlation between *Escherichia-Shigella* and serum C-reactive protein in weaning piglets became statistically significant in weaned piglets. Although a causal relationship between the abundance of gut microbiota and serum indices cannot be confirmed until more rigorous experiments (fecal microbiota transplantation) are done, our data provides a hint that gut microbiota structure at weaning is tightly related to post-weaning growth and well-being of piglets, and strengthened the opinion that post-weaning growth performance and healthy status can be intervened through modulating the maternal diet by way of piglets' gut microbiota⁽⁴⁶⁾.

It is worth mentioning that the establishment and maintenance of gut ecosystem in weaning piglets are determined by complex internal and external factors⁽³⁾, especially the microbiota from their moms⁽⁴⁷⁾. The intestine of neonates prior to birth is close to germ-free but rapidly shifts to a relatively complex

microbial population⁽³⁾, and the gut microbiota in piglets within 3 d after birth was predominantly affected by the vaginal microbiota of dams, then gradually affected by the sow fecal and slatted floor microbiota over time⁽⁴⁷⁾. It is a shortage of the current experiment that we missed the vaginal swabs from the sows in labour, cautious stool and slatted floor samples during lactation, thus it is hard to trace the direct target microbiota in response to maternal fish oil, especially those possessing probiotic effects in piglets during suckling-to-weaning transition.

Conclusion

Our study demonstrated that maternal administration of fish oil during late pregnancy and lactation could be passed to the offspring via colostrum, thereby elevating serum levels of IGF1, T3 and immunoglobulins, reducing diarrhoea in weaned piglets, which appear to be associated with alteration of the gut microbial community.

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The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit <https://doi.org/10.1017/S0007114522003981>

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