Effects of in ovo feeding and dietary addition oils on growth performance and immune function of broiler chickens

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ABSTRACT This study aimed to investigate the effects of in ovo feeding (**IOF**) and dietary addition (**DA**) oils on growth, development and immune function of broiler chickens. In experiment 1, a total of 500 eggs were randomly assigned to 3 treatments: non-injected group (**CON**) with 100 eggs; soybean oil injected group (**SO**) with 200 eggs and linseed oil injected group (**LO**) with 200 eggs. Results showed that there were no detrimental effects of IOF of oils on embryonic development. In experiment 2, a two factor experimental design was adopted. After hatching, 120 chicks which came from each oil-injected group were divided into 2 treatments with 6 replicates, and chickens were fed soybean oil diet and linseed oil diet, respectively. The results showed that DA linseed oil increased final body weight (**FBW**) of broilers at d 21 post hatch, IOF of linseed oil decreased average daily feed intake (**ADFI**) and feed conversion ratio (**FCR**) of broilers from d 1 to 21 ($P < 0.05$), while the plasma leptin level of 21-day-old broilers was increased by IOF or DA linseed oil ($P < 0.05$). Main effect analysis showed that DA linseed oil increased the spleen index and mRNA expression of *IFN-γ* in spleen of broilers at 7 d of age ($P < 0.05$). IOF of linseed oil upregulated the mRNA expression of *IFN-γ* in the spleen of chicks at 1 d and mRNA expression of *IL-2* and *IL-4* in spleen of broilers at 21 d ($P < 0.05$), and the interaction effect showed that IOF and DA linseed oil synergically increased the expression of *IL-2* and *IL-4* in spleen of broilers at 21 d. Compared with SO group, LO increased the Shannon index of hatching-day cecum microflora ($P < 0.05$). Principal co-ordinates analysis (**PcoA**) showed that LO group clearly separated from CON and SO groups. Finally, Spearman correlation analysis also manifested that *Alkalicoccus* was significantly correlated with spleen index and mRNA expression of *IL-2*, and *Phreatobacter* was significantly correlated with the mRNA expression of *IL-2* and *IFN-γ* in spleen, *Acinetobacter* had a positive correlation with thymus index ($P < 0.05$). In conclusion, IOF of linseed oil reduced the ADFI and FCR of broilers and increased the species diversity and changed the structure of cecal microflora of chicken embryos at the 19th day of incubation (E19). Immune function of broilers spleen was also regulated by IOF and DA linseed oil.

Key words: broiler chicken, immune function, intestinal microflora, in ovo injection, polyunsaturated fatty acid

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INTRODUCTION

In the process of poultry production, immune function impairment is caused by delayed feeding and long-distance transport, which may increase the susceptibility of poultry to various diseases and secondary infections and the risk of death (He et al., 2017). Therefore, the health status of chicks is a crucial factor that affects the growth and development of broilers in later period. Combining the intricately external factors and shorter incubation period, the nutrients only deposited in the egg may not fully satisfy the rapid growth and development of the body (Speake et al., 1998; Druyan, 2010). Consequently, regulating the early growth and development of broilers by in ovo injection of efficient nutrients has gradually become a vital focus. Numerous studies have shown that early nutritional intervention by in ovo feeding (**IOF**) can enhance the growth performance, promote the immune system development, and maintain the intestinal health of poultry (Ao et al., 2012; Roto et al., 2016; Jha et al., 2019).

Polyunsaturated fatty acid (**PUFA**) is a straight-chain fatty acid and can be divided into n-3 series and n-6 series. Polyunsaturated fatty acid enables to improve the growth performance and regulate the immune function of poultry. Most importantly, n-3 PUFA have anti-inflammation, immunoregulation, and antioxidation effects, while n-6 PUFA have adverse effects on immune function (Calder et al., 2019; Martindale et al., 2020).

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These opposite effects are mainly caused by the different inflammatory mediators that produced from the competitive metabolism (Brudvik and Taskén, 2011; Serhan, 2017; Serhan et al., 2018). The study also found that the growth of chick thymus, spleen and bursa was significantly affected by the ratio of n-6/n-3 PUFA and the composition of n-3 PUFA in the diet (Wang et al., 2000). Studies illuminated that increase the intake of n-3 PUFA can enrich its deposition in immune cells (Yaqoob et al., 2000; Rees et al., 2006), and enhance the concentration of anti-inflammatory medium in the blood (Pcca, 2020). Polyunsaturated fatty acid enables to activate the transcription factors and modulate gene expression by regulating signal transduction in immune cells (Miles et al., 2021).

Gut microbiota is a complex microbial ecosystem, which plays a critical role in host metabolism, immune response and physiological processes (Turnbaugh et al., 2006; Huttenhower et al., 2012; Ridaura et al., 2013). The fastest period for chick intestinal development is before and after the hatching period, and the colonization of early intestinal microorganisms play irreplaceable roles in maintaining and improving the intestinal health for entire life of broiler. N-3 polyunsaturated fatty acid can involve in intestinal immune regulation and maintain the intestinal homeostasis, which is closely related to intestinal microflora establishment and fatty acid metabolism (Costantini et al., 2017; Feng et al., 2020). Intake of n-3 PUFA augmented abundance of beneficial intestinal bacteria in the high-fat fed mice (Mujico et al., 2013). As for the poultry, retained nutrients in the yolk directly influence the embryo development. Based on these result, we speculate that in ovo injection of PUFA in the yolk sac will have great potential to influence the embryonic development and broilers performance. Meanwhile, the study shown that n-6 PUFA in soybean oil accounts for about 51% and n-3 PUFA is about 6.8% (Messina et al., 2021), while the linseed oil contains α-linolenic acid, which belongs to one of the n-3 PUFAs, more than 50% (Resende et al., 2021). Hence, this study was conducted to explore the effects of IOF and dietary supplement of soybean oil (rich in n-6 PUFA) or linseed oil (rich in n-3 PUFA) on performance and immune function of broilers from 19 d incubation to 21 d post-hatch.

MATERIALS AND METHODS

Ethics Statement

The protocol for the animal experimental procedures was approved by the Institutional Animal Care and Use Committee of Northwest A&F University (Permit Number: NWAFAC 1008).

Experiment 1

Fertilized Arbor Acres broiler eggs were obtained from the Xianyang Dacheng Poultry Co., Ltd. (Xianyang, China). Eggshells were sterilized with 1% bromegeramine solution and randomly assigned to 5 layers of the microcomputer automatic incubator (Dezhou Agricultural Science Incubation Equipment Co., Ltd., Dezhou, China). A total of 500 fertilized eggs with similar weight were randomly allocated to 3 groups: no injection group (**CON**) with 100 eggs; Soybean oil injection group (**SO**) with 200 eggs; Linseed oil injection group (**LO**) with 200 eggs. Before the injection, the eggs were candled for selecting embryonated eggs, and the unfertilized or nonviable eggs were removed. At 11 d of incubation, 0.2 mL of oil was injected into the embryonic eggs. The oil was slowly injected into the yolk sac with a 1 mL sterilizing syringe and the inserted depth was almost 2 cm. Injection method and site were referred to previous publication (Zhu et al., 2019). Although the control group was non-injected, it was subjected to the same handling procedures as the other treatment groups.

At E19 and the day of hatch, 6 live embryonated eggs and chicks were selected from each treatment. The embryo body weight (**EBW**, included yolk), and absolute body weight (removed yolk) of each selected egg and chick were determined. The cecal tissue was also collected for 16S rDNA sequencing at E19. The hatching rate of chicks was also recorded.

Experiment 2

In the feeding stage, a 2 × 2 experimental design was conducted. After hatching, 120 chicks from each oil-injected group were selected and housed in single layer cages. Chicks, which came from SO and LO injected group, were then fed with soybean oil diet and linseed oil diet, respectively. Namely, the 240 chickens were divided into 4 treatments: SO-SO group, SO-LO group, LO-SO group, and LO-LO group. Each treatment had 6 replicates, and 10 birds of each replicate were evenly distributed in the broiler house. The temperature in the chick house was maintained at 35°C for the first week and gradually decreased to 27°C in the third wk. The light was provided 23 h per day and the chicks had free access to feed and water. The ingredients and composition of the experimental diets are shown in Table 1. Body weight (**BW**), average daily food intake (**ADFI**), average daily weight gain (**ADG**), and FCR were recorded during the experimental phase.

At post-hatch day 1 (**D1**), 7 and 21, one bird from each replicate was randomly selected and weighed, blood collected and euthanized by cervical dislocation. The intact spleen, thymus, and bursa were quickly dissected, weighed, and recorded, frozen in liquid nitrogen, and then transferred to −80°C refrigerator for further analysis.

Samples Processing and Laboratory Analyses

DNA extraction, which obtained from cecal samples of chicken embryos at E19 in experiment 1, was carried out using the (**OMEGA**) stool DNA Kit (D4015-02; Omega

Table 1. Ingredient and nutrient composition of experimental diets.

Ingredients (%)	Soybean oil diet	Linseed oil diet
Corn	60.66	60.66
Soybean meal	28.84	28.84
Cottonseed meal	4.00	4.00
Soybean oil	2.50	—
Linseed oil	—	2.50
Calcium hydrogen phosphate	1.52	1.52
Limestone	1.40	1.40
Salt	0.30	0.30
DL-Met	0.16	0.16
L-Lys	0.27	0.27
L-Thr	0.10	0.10
Microelement[1]	0.10	0.10
Multivitamin[2]	0.03	0.03
Choline chloride	0.10	0.10
Antioxidant	0.02	0.02
Nutrient levels		
ME (MJ/kg)	12.34	12.37
CP	21.00	21.00
Available lysine	1.25	1.25
Available methionine	0.49	0.49
Ca	1.00	1.00
Available P	0.39	0.39

[1]The microelement provided the following per kg of diets: Cu 8.7 mg, Zn 85 mg, Fe 60 mg, Mn 63 mg, Se 0.36 mg, I 1.0 mg.

[2]The multivitamin provided the following per kg of diets: vitamin A 9,000 IU, vitaminD_3 ,3,000 IU, vitamin E 36 IU, vitamin K_3 2.7 mg, vitamin B_1 3.6 mg, vitamin B_2 7.5 mg, vitamin B_6 5.4 mg, vitamin B_{12} 36 mg, biotin 210 mg, folic acid 1.5 mg, pantothenic acid 15 mg, nicotinic acid 51 mg.

Bio-tek, Norcross, GA). PCR was performed to amplify the V4-V5 region of the 16S rRNA gene, using 515 F (forward primer) 5′-GTGCCAGCMGCCGCGGG)-3′ and 907 R (reverse primer) 5′-CCGTCAATTCMTTTRAGTTT-3′ as sample-specific sequence barcoded fusion primers. The PCR products were purified, quantified, and sequenced using Illumina HiSeq PE250. 16S rRNA sequencing was carried out at Shanghai Ling 'en Biotechnology Limited Company, Shanghai, China, the bioinformatics analysis was conducted according to our previous introduction (Yang et al., 2020).

The plasma samples form experiment 2 were used to determined leptin level and other plasma index under the recommended procedures of a commercially available kit from the Nanjing Aoqing Biotechnology Limited Company (ANG-E32151C; Nanjing, China). The lysozyme (**LZM**) contents in plasma were measured using a commercially available kit from Nanjing Jiancheng Bioengineering Institute (A050; Nanjing, China), based on the turbidimetric method. The contents of total protein (**TP**), albumin (**ALB**), and globulin (**GLO**) in plasma were determined by the automatic biochemical analyzer.

Spleen tissue samples from experiment 2 were used to measure expression of cytokine. The total RNA was extracted using the Trizol Reagent Kit (Accurate Biotechnology Co., Ltd, Changsha, China) according to the manufacturer's instructions. Reverse transcription of cDNA using Evo-M-MLV reverse transcription reagent premix (Accurate Biotechnology Co., Ltd). The real-time quantitative PCR reaction was performed using SYBR Green Pro Taq HS Premixed Kit (Accurate Biotechnology Co., Ltd). The detailed procedures were based on previous description (Wang et al., 2019b). Primers for internal control genes (***β-actin***) and interferon gamma (***IFN-γ***) were designed using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/), and primers for interleukin-2 (***IL-2***) interleukin-4 (***IL-4***) were referenced to Li et al. (2016). The primer sequences were listed in Table S1.

Statistical Analysis

The SPSS 26.0 statistical software (SPSS Inc., Chicago, IL) was used for all statistical analyses in this study. For experiment 1, the data of embryonic stage were analyzed by one-way ANOVA. For experiment 2, the data of feeding stage was analyzed using Univariate analysis to assess the main effects of in ovo injection and dietary addition of oil and their interaction. Tukey's range test was used to detect significant differences between individual means. The results were expressed as mean ± SEM, and $P < 0.05$ was implied a significant difference.

RESULTS

Experiment 1

Embryonic Growth The results showed that the hatchability of each group was more than 85%, and there were no significant differences between groups (shown in Supplementary Table S2). The results of IOF of oils on the body weight of embryos at E19 and chicks at 1-day-old are presented in Table 2. IOF of soybean oil and linseed oil had no effects on the body weight and absolute body weight of embryos at E19 and chicks at 1 d of age ($P > 0.05$).

Immune Organ Index and Plasma Index of the Embryos and Chicks The immune organ index and plasma index of embryos and 1-day-old broilers are presented in Table 3. At d 19 of incubation and 1 d of age, no significant effects were found in spleen index, thymus index, and bursa index among each treatment ($P > 0.05$). Contents of plasma LZM, TP, ALB, and GLO were changeless among groups ($P > 0.05$).

mRNA Expression of Cytokine in the Embryo and Chicks Spleen The results of IOF and DA oils on mRNA expression of *IL-2, IL-4*, and *IFN-γ* in spleen are

Table 2. Effects of in ovo feeding of oils on the body weight at E19 and D1.

Items[1]		Treatments			SEM	*P*-value
		CON	SO	LO		
E19	Embryo weight/g	42.84	41.68	40.80	0.75	0.589
	Absolute weight/g	28.47	28.58	27.96	0.74	0.939
D1	Body weight/g	44.98	44.93	45.18	0.49	0.978
	Absolute weight/g	38.04	37.96	38.09	0.40	0.992

The results are represented as the mean value with pooled SEM (n = 6).

[1]Abbreviations: CON, non-injected group; D1, first day of hatching; E19, 19th day of incubation; LO, linseed oil injected group (eggs injected with 0.2 mL of linseed oil at E11); SO, soybean oil injected group (eggs injected with 0.2 mL of soybean oil at E11).

Table 3. Effects of in ovo feeding of oils on the immune organ index and plasma index at E19 and D1.

Items[1]			Treatments			SEM	*P*-value
			CON	SO	LO		
Immune organ index	E19	Spleen (g/kg)	0.45	0.46	0.46	0.02	0.982
		Thymus (g/kg)	1.09	1.09	1.04	0.08	0.953
		Bursa (g/kg)	1.21	1.35	1.09	0.07	0.252
	D1	Spleen (g/kg)	0.32	0.35	0.33	0.02	0.760
		Thymus (g/kg)	0.91	1.29	1.08	0.09	0.255
		Bursa (g/kg)	1.13	1.22	1.33	0.07	0.515
Plasma index	D1	LZM (U/mL)	204.43	205.26	221.86	15.38	0.882
		TP (g/L)	24.92	21.20	22.88	0.83	0.190
		ALB (g/L)	9.43	8.28	8.83	0.31	0.331
		GLO (g/L)	15.48	12.92	14.05	0.62	0.256

The results are represented as the mean value with pooled SEM (n = 6).
[1]Abbreviations: CON, non-injected group; SO, soybean oil injected group (eggs injected with 0.2 mL of soybean oil at E11); LO, linseed oil injected group (eggs injected with 0.2 mL of linseed oil at E11); LZM, lysozyme; TP, total protein; ALB, albumin; GLO, globulin.

shown in Table 4. IOF of oils had no significant effects on the mRNA expression of *IL-2, IL-4*, and *IFN-γ* in spleen of chicks at E19 ($P > 0.05$). Compared with CON group, IOF of linseed oil significantly up-regulated the mRNA expression of *IFN-γ* in spleen of chicks at 1 d of post hatch ($P < 0.05$).

Cecal Microbiota of Embryos and Correlation Analysis According to the Venn diagram (Figure 1A), there were 177 common OTUs among the 3groups, 92 unique OTUs in the CON group, 43 unique OTUs in the SO group, and 156 unique OTUs in the LO group. Shannon index (Figure 1B) in LO group was significantly higher compared with SO group ($P < 0.05$), and had a gentle increase than that in CON group ($P = 0.053$). According to results of PCoA analysis (Figure 1C), the LO group was significantly separated from other groups, and suggesting that the difference in species composition was caused by IOF of linseed oil. The compositions of chick gut microbiota of each group at the phylum and genus levels were shown in Figures 1D and 1E. Unfortunately, 2 samples in the SO group were polluted, so we discarded them from the data analysis. There was no remarkable difference in species abundance at the phylum level of embryo cecum at E19 (Table 5). *Proteobacteria, Bacteroidetes*, and *Actinobacteria* were the most abundant microorganisms in each group. At the genus level, *Labrys, Mesorhizobium*, and *Vibrionimonas* were the dominant bacteria in each group. For the relative abundance of top 15 species (Table 5), results showed that the relative abundance of *Ralstonia* in SO group was apparently lower than that in CON group ($P < 0.05$), but there was no significant difference between LO group and CON group ($P > 0.05$).

Lefse analysis clarified the differences of flora among groups, and linear discriminant analysis (LDA SCORE) was used to estimate the abundance differences of each species (Figures 1F and 1G). As for the enriched differential bacteria, the CON group included *Acidovorax, Erysipelotrichaceae* and *Muribaculaceae*, while the SO group only had *Nitriliruptor*. And the LO group had more enriched differential bacteria, including *Xanthomonadaceae, Pelagibacterium, Soilbacillus, Herbaspirillum, Gemmobacter, Sporolactobacillaceae, Sphingomonadaceae, Propionibacteriaceae, Alkalicoccus*, etc. As shown in Figure 1H, the Spearman correlation coefficient was used to evaluate the correlation between the abundance of top 50 bacteria in cecum and immune indices of embryos at E19. *Nocardia, Pelomonas, Nesterenkonia, Aliidiomarina, Alkalicoccus*, and *Bosea* were positively correlated with spleen index ($P < 0.05$). Thymus index had a positive correlation with *Acinetobacter* ($P < 0.05$). The relative expression of splenic cytokine *IL-2* and *IL-4* were both positively correlated with *Phreatobacter* ($P < 0.05$). Furthermore, the abundance of *Lactobacillus* was positively correlated with mRNA expression of *IL-4* and *IFN-γ* in the spleen ($P < 0.05$).

Table 4. Effects of in ovo feeding and dietary addition oils on relative mRNA expression of cytokines in spleen of broilers at E19 and D1.

Items[1]		Treatments			SEM	*P*-value
		CON	SO	LO		
E19	*IL-2*	1.00	0.74	1.35	0.15	0.234
	IL-4	1.00	0.85	0.97	0.09	0.776
	IFN-γ	1.00	0.75	1.13	0.11	0.351
D1	*IL-2*	1.00	0.90	1.26	0.11	0.431
	IL-4	1.00	0.99	1.43	0.14	0.327
	IFN-γ	1.00[b]	1.15[ab]	1.66[a]	0.12	0.044

The results are represented as the mean value with pooled SEM (n = 6).
[1]Abbreviations: CON, non-injected group; LO, linseed oil injected group (eggs injected with 0.2 mL of linseed oil at E11); SO, soybean oil injected group (eggs injected with 0.2 mL of soybean oil at E11); *IL-2*, interleukin-2; *IL-4*, interleukin-4; *IFN-γ*, interferon gamma.
[a,b]Means within a row without common superscript differ significantly ($P < 0.05$) among the groups.

Experiment 2

Growth Performance and Leptin Levels in Plasma of Broilers Table 6 shows the initial body weight (**IBW**), final body weight (**FBW**), ADG, ADFI, and FCR of broilers from 1 to 21 d of post hatch. No significant differences were observed in the IBW of broilers among all treatments ($P > 0.05$), and the FBW of broilers was increased by DA linseed oil ($P < 0.05$). IOF of linseed oil decreased the ADFI and FCR of broilers during 1 to 21 d ($P < 0.05$), but had no effect on the average daily gain ($P > 0.05$). There was no interaction between IOF and DA on performance of broilers ($P > 0.05$). Plasma leptin level was measured based on the

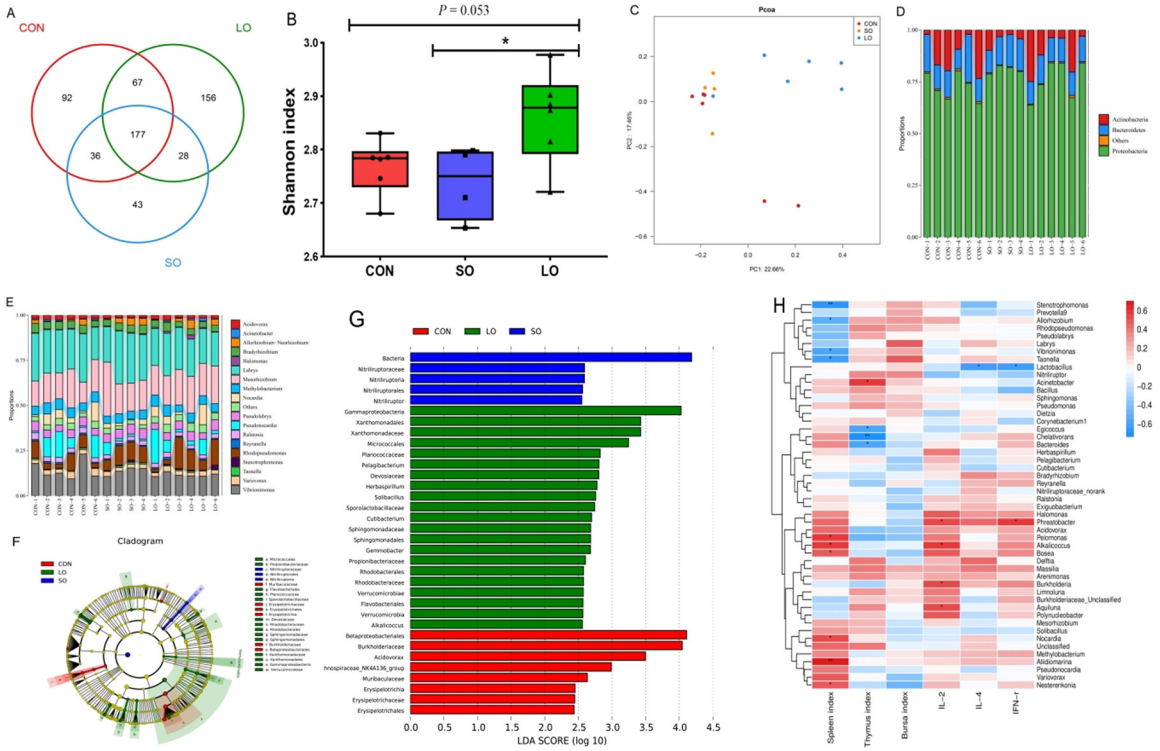

Figure 1. Effects of in ovo feeding oils on cecal microbiota of embryos at E19. (A) The Venn diagram of OTU, (B) Alpha diversity indexes (Shannon) of the cecal microbiota, (C) PCoA diagram based on the unweighted UniFrac distances of beta diversity of the cecal microbiota, (D) cecal microbial compositions at phylum level (Only phyla with an average abundance greater than 1% were included), and (E) cecal microbial compositions at genus level (Only genus with an average abundance greater than 1% were included). (F) Taxonomic cladogram generated from LEfSe showing significant difference in the microbiota profile of 3 groups, and (G) linear discriminant analysis (LDA SCORE) to estimate the magnitude of difference in abundance of each species. (H) The Spearman correlation analysis among cecal top 50 genera and immune indices. The results are expressed as the mean and pooled SEM (CON = 6, SO = 4, LO = 6). * Means differ significantly ($P < 0.05$) among the groups. Abbreviations: CON, non-injected group; LO, linseed oil injected group (eggs injected with 0.2 mL of linseed oil at E11); SO, soybean oil injected group (eggs injected with 0.2 mL of soybean oil at E11).

difference ADFI. The plasma leptin of chicks at 1 d was increased by IOF of linseed oil ($P = 0.093$, shown in Supplementary Table S3). It can be seen from Table 6 that IOF and DA linseed oil increased the plasma leptin content of broilers at 21-day-old ($P < 0.05$), and there was no interaction between IOF and DA ($P > 0.05$).

Immune Organ Index and Plasma Index of Broilers

The immune organ index and plasma index of broilers at

Table 5. The relative abundance of cecal microbial flora at phylum and genus level of embryos at E19.

Items[1]		Treatments			SEM	*P*-value
		CON	SO	LO		
Phylum percent	*Proteobacteria*	72.58	80.84	76.14	1.84	0.230
	Bacteroidetes	14.39	13.87	11.85	0.86	0.437
	Actinobacteria	12.28	4.92	11.40	2.09	0.376
	Firmicutes	0.42	0.27	0.36	0.03	0.181
Genu percent	*Labrys*	23.52	25.45	20.98	0.86	0.116
	Mesorhizobium	15.93	19.59	18.54	1.04	0.368
	Vibrionimonas	14.24	13.72	11.57	0.87	0.403
	Rhodopseudomonas	5.60	8.91	8.71	1.16	0.444
	Pseudonocardia	7.50	2.64	6.60	1.41	0.410
	Methylobacterium	5.41	4.72	5.14	0.19	0.411
	Pseudolabrys	4.44	5.26	4.54	0.20	0.241
	Bradyrhizobium	4.24	4.14	3.70	0.16	0.327
	Nocardia	4.39	1.87	4.02	0.84	0.511
	Variovorax	2.93	2.27	2.42	0.17	0.273
	Ralstonia	2.83[a]	2.07[b]	2.33[ab]	0.13	0.044
	Acidovorax	2.10	1.46	1.91	0.11	0.055
	Allorhizobium	1.20	2.65	1.84	0.40	0.405
	Reyranella	0.85	0.81	0.84	0.05	0.952
	Taonella	0.58	0.80	0.55	0.06	0.192

The results are expressed as the mean and pooled SEM (CON = 6, SO = 4, LO = 6).

[1]Abbreviations: CON, non-injected group; LO, linseed oil injected group (eggs injected with 0.2 mL of linseed oil at E11); SO, soybean oil injected group (eggs injected with 0.2 mL of soybean oil at E11).

[a,b]Means within a row without common superscript differ significantly ($P < 0.05$) among the groups.

Table 6. Effects of in ovo feeding and dietary addition oils on the performance during D1−21 and leptin concentration in plasma of broilers at D21.

Items[1]		IBW/g	FBW/g	ADFI/g	ADG/g	FCR	Leptin (ng/mL)
Treatments	SO-SO	43.57	575.10	34.33	23.61	1.45	8.36
	SO-LO	43.60	640.16	39.31	26.11	1.50	10.41
	LO-SO	43.84	593.43	29.55	23.88	1.23	11.50
	LO-LO	43.75	614.49	30.36	24.03	1.26	12.47
SEM	0.21	8.28	1.17	0.40	0.03	0.25	
Main effect							
IOF	SO	43.58	607.631	36.82a	24.86	1.48a	9.38b
	LO	43.79	603.96	29.95b	23.96	1.25b	11.98a
DA	SO	43.70	584.26b	31.94	23.74	1.34	9.93b
	LO	43.68	627.32a	34.83	25.07	1.38	11.44a
P-value	IOF	0.648	0.797	0.001	0.228	<0.001	<0.001
	DA	0.954	0.006	0.128	0.083	0.429	<0.001
	IOF × DA	0.896	0.133	0.265	0.123	0.826	0.140

The results are represented as the mean value with pooled SEM (n = 6).

[1]Abbreviations: SO-SO, *in ovo* feeding of soybean oil and dietary addition soybean oil; SO-LO, *in ovo* feeding of soybean oil and dietary addition linseed oil; LO-SO, *in ovo* feeding of linseed oil and dietary addition soybean oil; LO-LO, *in ovo* feeding of linseed oil and dietary addition linseed oil; IOF, *in ovo* feeding of oils; DA, dietary addition of oils; IOF×DA, the interaction between IOF of oils and DA of oils; IBW, the initial weight of hatchling; FBW, the final weight of broilers at D21.

[a,b]Means within a row without common superscript differ significantly ($P < 0.05$) among the groups.

7 and 21 d of age are presented in Table 7. Dietary linseed oil increased spleen index of broilers at 7 d ($P < 0.05$); IOF and DA oils had no remarkable influence on spleen index, thymus index, and bursa index of broilers at 21 d ($P > 0.05$). No interaction was seen between IOF and DA at 7 d and 21 d of age ($P > 0.05$). As for the plasma index, all treatments had no significant effects on the contents of plasma LZM, TP, ALB and GLO of broilers at D7 and D21 ($P > 0.05$). There was no obvious interaction between IOF and DA on plasma index of broilers at 7 d and 21 d of age ($P > 0.05$).

mRNA Expression of Cytokine in the Broilers Spleen As shown in Table 8, Dietary linseed oil increased the mRNA expression of *IFN-γ* in spleen of broilers at 7 d ($P < 0.05$), and results showed that IOF and DA had no interaction effects on expression of *IFN-γ* ($P > 0.05$). For 21-day-old broilers, IOF of linseed oil upregulated the mRNA expression of *IL-2* and *IL-4* in spleen ($P < 0.05$), and the interaction effect showed that IOF and DA linseed oil synergistically increased the expression of *IL-2* and *IL-4* ($P < 0.05$).

DISCUSSION

Based on our review of the literature, there is a dearth of information in the literature that evaluated the effects of IOF oils on broiler chicks. The different effects of dietary oil (rich in PUFA) on poultry performance vary from the dose, type, and composition. Long et al. (2020) showed that compared with the diet supplemented with soybean oil, the diets supplemented with microalgae, linseed oil, and fish oil significantly increased the ADG and reduced the FCR of broilers from d 1 to 21. Chen et al. (2014) showed that dietary supplemented with 15% linseed oil significantly improved the FCR and laying rate of geese, also indicating that an appropriate portion of n-3 PUFA can improve the performance of laying geese. Nutritional interfering is the indispensable means for scientific research during the growth process of broiler. Given that the embryonic period and the post-hatching growth period co-affect the broiler's performance, in our research, we take account of the cumulative effect about in ovo feeding and dietary addition of oils on the broiler growth performance and immune function. In the current study, it was found that the average hatching rate between the IOF group and the CON group was 86.83%, and no significant difference was observed. There were no significant changes on weight and absolute weight in embryos at E19 and chicks at 1-day-old, indicating that the injection of soybean oil and linseed oil had no negative effect on embryo development. In the feeding stage, the dietary addition linseed oil increased the FBW, and IOF of linseed oil reduced the ADFI and FCR of broilers. This is not consistent with previous studies on dietary linseed oil supplementation. The inconsistent effects of linseed oil on performance may be attributed to the intestinal function (Boudry et al., 2009; Abdulla, 2016), and the further research is needed to tease out the specific reason. Leptin is a protein hormone. After entering the bloodstream, it can participate in the regulation of energy metabolism and inhibit the appetite. Denbow et al. (2000) reported that injecting the protein of human recombinant leptin into the broiler ventricle could sharply reduce the feed intake. Studies also shown that leptin significantly inhibits the mRNA expressions of neuropeptide Y (**NPY**), orexin (**ORX**) and other genes in the hypothalamus of broilers at 21d, thereby inhibiting the feeding intake of poultry (Dridi et al., 2005). Previous studies manifested that linoleic acid and arachidonic acid could affect the leptin signaling by inducing leptin resistance with no significant changes in body weight (Cheng et al., 2015). In accordance with the previous research results, IOF of soybean oil increased the ADFI, while linseed oil supplementation significantly increased leptin level at 21 d of age. The elevated leptin level was the main reason that IOF of linseed oil induced a decrease on feed intake of broilers. Interestingly, we observed that the average daily feed intake of broilers in

Table 7. Effects of in ovo feeding and dietary addition oils on immune organ index and plasma index of broilers at D7 and D21.

Items[1]		Immune organ index						Plasma index							
		D7			D21			D7			D21				
		Spleen index	Thymus index	Bursa index	Spleen index	Thymus index	Bursa index	LZM (U/mL)	TP (g/L)	ALB (g/L)	GLO (g/L)	LZM (U/mL)	TP (g/L)	ALB (g/L)	GLO (g/L)
Treatments	SO-SO	0.61	1.49	1.63	0.96	2.24	2.73	278.23	25.58	12.13	13.45	253.80	25.58	13.55	12.03
	SO-LO	0.67	1.89	1.62	0.80	2.32	2.17	269.66	25.62	11.88	13.73	233.33	25.73	13.18	12.55
	LO-SO	0.49	1.25	1.60	0.76	2.21	2.33	279.46	26.52	12.12	14.40	225.17	25.35	13.48	11.87
	LO-LO	0.73	1.32	1.82	0.75	2.27	2.65	275.36	26.33	12.20	14.13	226.20	26.27	13.55	12.72
SEM		0.11	0.09	0.04	0.14	0.12	3.93	0.43	0.19	0.27	18.79	0.39	0.19	0.23	
Main effect															
IOF	SO	0.64	1.69	1.63	0.88	2.28	2.45	273.94	25.60	12.01	13.59	243.56	25.66	13.37	12.29
	LO	0.61	1.28	1.71	0.76	2.24	2.49	277.41	26.43	12.16	14.27	225.69	25.81	13.52	12.29
DA	SO	0.55[b]	1.37	1.62	0.86	2.23	2.53	278.84	26.05	12.13	13.93	239.48	25.47	13.52	11.95
	LO	0.70[a]	1.60	1.72	0.77	2.29	2.41	272.51	25.98	12.04	13.93	229.77	26.00	13.37	12.63
P-value	IOF	0.582	0.064	0.666	0.100	0.886	0.873	0.679	0.371	0.716	0.235	0.660	0.856	0.714	1.000
	DA	0.012	0.272	0.570	0.233	0.826	0.633	0.452	0.934	0.840	0.988	0.810	0.522	0.714	0.166
	IOF × DA	0.115	0.439	0.528	0.310	0.969	0.085	0.789	0.905	0.686	0.624	0.791	0.645	0.597	0.729

The results are represented as the mean value with pooled SEM (n = 6).

[1]Abbreviations: SO-SO, *in ovo* feeding of soybean oil and dietary addition soybean oil; SO-LO, *in ovo* feeding of soybean oil and dietary addition linseed oil; LO-SO, *in ovo* feeding of linseed oil and dietary addition soybean oil; LO-LO, *in ovo* feeding of linseed oil and dietary addition linseed oil; IOF, *in ovo* feeding of oils; DA, dietary addition of oils; IOF×DA, the interaction between IOF of oils and DA of oils; LZM, lysozyme; TP, total protein; ALB, albumin; GLO, globulin.

[a,b]Means within a row without common superscript differ significantly ($P < 0.05$) among the groups.

SO-LO group was higher than other groups. This result indicated that IOF of soybean oil perhaps played crucial roles in regulation of food intake and its metabolites probably could inhibit the body's sensitivity to leptin and consequently promote the feed intake. Therefore, we believe that n-3 PUFA is different from n-6 PUFA in regulating feeding intake of broilers, and the specific regulation mechanism needs to be further investigated.

The spleen, thymus and bursa are the main immune organs of poultry. The changes of the index of immune organs can basically reflect the development of the immune system. The mature immune system can effectively inhibit the invasion of pathogens. The results showed that IOF of oils had no significant effect on the development of immune organs of broilers at all ages. But dietary addition linseed oil significantly increased spleen index of 7-day-old broilers, indicating that dietary linseed oil had definitely promoted effect on spleen development of broilers.

Lysozyme is secreted by macrophages and multinucleated granulocytes. Once pathogens invading, lysozyme cleans bacteria mainly by dissolving the peptidoglycan on the surface of bacteria (Zhang et al., 2005), and this ability can reflect the body's nonspecific immune function. The total protein in plasma can reflect the absorption and utilization of protein, and the albumin in plasma is a reliable parameter to reflect the nutritional status of the body (Schmilovitz-Weiss et al., 2006). The globulin in plasma is mainly composed of various components such as immunoglobulin and complement, which can reflect the immune function of the body to some extent. Ibrahim et al. (2018) showed that dietary supplementation of fish oil and linseed oil significantly increased serum lysozyme activity of laying hens, while dietary supplementation with corn oil significantly decreased lysozyme activity. This shows that dietary PUFA content and type may have an impact on macrophage activation and antigen presentation. However, after hatching, the lysozyme activity and plasma protein content were stabilized among groups. This may indicate that the immune function of broilers maintained in balance.

The cytokines play an important role in body's immune response by activating immunoreactive (Collison et al., 2007). *IL-2*, which is secreted by Th1 cells, not only promotes T cell proliferation, but also plays a key role in regulating the stability of Treg cell (Boyman and Sprent, 2012; Morgan et al., 1976). *IFN-γ* secreted by Th1 cells and could promote the proliferation, differentiation and maturation of T cells, and improve the activity of NK cells and the phagocytosis of monocyte macrophages (Chan et al., 2015). *IL-4* secreted by Th2 cells is involved in regulating the proliferation, differentiation and maturation of B cells (Swain et al., 1990). Therefore, the changes of mRNA expression of *IL-2, IL-4, IFN-γ*, and other cytokines in the spleen can be used to reflect the regulatory function of immune system. It has been shown that reducing the ratio of n-6/n-3 PUFA in the dietary could increase the relative mRNA expression of *IFN-γ, IL-1β, IL-2*, and *IL-6* (Ibrahim et al., 2018). In this study, we found that IOF

Table 8. Effects of in ovo feeding and dietary addition oils on relative mRNA expression of cytokines in spleen of broilers at D7 and D21.

		D7			D21		
Items[1]		*IL-2*	*IL-4*	*IFN-γ*	*IL-2*	*IL-4*	*IFN-γ*
Treatments	SO-SO	1.01	1.02	1.04	1.04[b]	1.09[b]	1.01
	SO-LO	1.45	1.24	1.34	0.87[b]	0.85[b]	0.79
	LO-SO	1.19	1.20	1.12	1.17[b]	0.98[b]	0.92
	LO-LO	1.24	1.07	1.96	1.70[a]	1.58[a]	1.16
SEM		0.06	0.05	0.12	0.08	0.09	0.07
Main effect							
IOF	SO	1.23	1.13	1.19	0.96[b]	0.97[b]	0.90
	LO	1.21	1.13	1.54	1.43[a]	1.28[a]	1.04
DA	SO	1.10	1.11	1.08[b]	1.10	1.03	0.97
	LO	1.34	1.16	1.65[a]	1.28	1.21	0.97
P-value	IOF	0.900	0.970	0.078	0.000	0.030	0.295
	DA	0.052	0.667	0.007	0.117	0.193	0.959
	IOF × DA	0.115	0.128	0.168	0.006	0.006	0.093

The results are represented as the mean value with pooled SEM (n = 6).

[1]Abbreviations: SO-SO, *in ovo* feeding of soybean oil and dietary addition soybean oil; SO-LO, *in ovo* feeding of soybean oil and dietary addition linseed oil; LO-SO, *in ovo* feeding of linseed oil and dietary addition soybean oil; LO-LO, *in ovo* feeding of linseed oil and dietary addition linseed oil; IOF, *in ovo* feeding of oils; DA, dietary addition of oils; IOF×DA, the interaction between IOF of oils and DA of oils; *IL-2*, interleukin-2; *IL-4*, interleukin-4; *IFN-γ*, interferon gamma.

[a,b]Means within a row without common superscript differ significantly ($P < 0.05$) among the groups.

of linseed oil upregulated the mRNA expression of *IFN-γ* in spleen of chicks at 1-day-old, and also upregulated the mRNA expression of *IL-2* and *IL-4* in spleen at 21 d. Dietary linseed oil also upregulated the expression of *IFN-γ* in spleen of broilers at 7 d of age. IOF and DA linseed oil showed synergistic effect on the expressions of *IL-2* and *IL-4* in the spleen of broilers at 21 d. These positive results indicated that both IOF and DA linseed oil could improve the immune function of broilers, which might partially due to the beneficial effects of α-linolenic acid and its derivatives in linseed oil.

Intestinal microorganisms not only play an important role in nutrient digestion and absorption, but also are closely related to the establishment of the immune system (Wang et al., 2019a). Since the embryo development completely separate from their mother, it is a controversial question that whether the chicken embryo contains microbes or not. Recent studies have shown that gut microbes colonized at the incubation stage, and chicken embryo microbes partially obtained from the fallopian tube and cloaca of the laying hens (Ding et al., 2017; Lee et al., 2019), and the coremicrobiota in embryo was similar with the maternal hen's gut (Akinyemi et al., 2020). Status of intestinal flora that established at incubation period is crucial for maintaining and improving intestinal health in the later growth period of broilers. Yolk is connected with the intestine at late hatching stage, so the nutrients retained in the yolk will influence the microbes forming (Ding et al., 2022). It was demonstrated that n-3 PUFA involved in the intestinal immune regulation and maintenance of intestinal homeostasis (Robertson et al., 2016; Costantini et al., 2017). In this research, Shannon index and PCoA analysis results showed that IOF of linseed oil increased the species diversity and modulated the intestinal microbial communities of embryos at E19. Calder and Philip (2017) also showed that dietary supplement of fish oil significantly affected the diversity of intestinal microorganism compared with sunflower seed oil. Results of this study showed that the dominant phyla of chicken embryos were *Proteobacteria, Bacteroidetes, Actinobacteria*, and *Firmicutes*, which was similar to the results of Akinyemi et al. (2020). As for the structure of the genus, IOF of soybean oil reduced the abundance of *Ralstonia. Ralstonia*, which belongs to the family of *Pseudomonadaceae,* is an aerobic type of Gram-negative nonfermentative bacteria and can cause diseases and infections under certain conditions (Ryan and Adley, 2014). In this experiment, LEfSe analysis was used to enrich differential bacteria in each group with LDA score greater than 2.0, and more differential bacteria were enriched in LO group. Among them, *Propionibacteriaceae* can ferment carbohydrates and produce propionic acid, acetic acid or a mixture compound of organic acids including butyric acid, formic acid, and lactic acid. *Sporolactobacillaceae* is involved in metabolic pathways of acetic acid and lactic acid (Park et al., 2019). Short-chain fatty acids act as energy source of intestinal epithelial cells (Donohoe et al., 2011), which can promote the development of intestinal villi, regulate intestinal morphology, and structure of broilers (Shakouri et al., 2009).

The correlation analysis between immune parameters with intestinal microorganism was observed in present study. *Lactobacillus* is a dominant bacterium in the intestinal tract and produces the beneficial substance such as lactic acid and involve in the immune function (Barbieri et al., 2019; Wu et al., 2019). Thus, it is reasonable that abundance of *Lactobacillus* was positively correlated with splenic cytokine. There were significant correlations between several bacterial genera and spleen index, thymus index, the mRNA expression of cytokine. This result indicated that the changes of intestinal microorganism during embryonic stage may be involved in the immune modulation but variation of microflora was not the principal factor for modulating the embryonic immune.

CONCLUSIONS

In conclusion, these results implied that in ovo feeding of linseed oil participated in the regulation of intestinal

microbes of chicken embryos, and exerted beneficial effects on performance of post-hatch broilers. Additionally, in ovo feeding and dietary addition linseed oil could co-regulate the immune function of broilers. This finding may imply the immunomodulatory effect of linseed oil, which is meaningful for the immune response of broiler chickens after vaccination. It would be also important and interesting to investigate the additional and specific functions of high purity of n-3 PUFA on chick embryo and broiler growth.

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DISCLOSURES

All authors declare no conflict of interests for this manuscript.

SUPPLEMENTARY MATERIALS

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REFERENCES

Abdulla, N. R. 2016. Growth performance and meat quality of broiler chickens supplemented with different oil sources. PhD Diss. Univ. Putra Malaysia, Selangor, Malaysia.

Akinyemi, F. T., J. Ding, H. Zhou, K. Xu, C. He, C. X. Han, Y. M. Zheng, H. X. Luo, K. X. Yang, C. Gu, Q. Z. Hang, and H. Meng. 2020. Dynamic distribution of gut microbiota in chickens with embryonic development. Poult. Sci. 99:5079–5090.

Ao, Z., A. Kocher, and M. Choct. 2012. Effects of dietary additives and early feeding on performance, gut development and immune status of broiler chickens challenged with clostridium perfringens. Asian-Austral. J. Anim. 25:541–545.

Barbieri, F., C. Montanari, F. Gardini, and G. Tabanelli. 2019. Biogenic amine production by lactic acid bacteria: a review. Foods 8:17.

Boudry, G., V. Douard, J. Mourot, J.-P. Lalles, and I. L. Huerou-Luron. 2009. Linseed oil in the maternal diet during gestation and lactation modifies fatty acid composition, mucosal architecture, and mast cell regulation of the ileal barrier in piglets. J. Nut. 139:1110–1117.

Boyman, O., and J. Sprent. 2012. The role of interleukin-2 during homeostasis and activation of the immune system. Nat. Rev. Immunol. 12:180–190.

Brudvik, K. W., and K. Taskén. 2011. Modulation of T cell immune functions by the prostaglandin e2-camp pathway in chronic inflammatory states. Brit. J. Pharmacol. 166:411–419.

Calder, P. C., L. W. Dan, S. Klek, and R. G. Martindale. 2019. Lipids in parenteral nutrition: biological aspects. J. Parenter. Enteral Nutr. 44:S21–S27.

Calder, P. C., and C. Philip. 2017. Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochem. Soc. T. 45:1105–1115.

Chan, A., T. Magnus, and R. Gold. 2015. Phagocytosis of apoptotic inflammatory cells by microglia and modulation by different cytokines: mechanism for removal of apoptotic cells in the inflamed nervous system. Glia 33:87–95.

Chen, W. S., Y. Y. Jiang, J. P. Wang, Y. Q. Huang, and Z. X. Wang. 2014. Effects of dietary flaxseed meal on production performance, egg quality, and hatchability of huoyan geese and fatty acids profile in egg yolk and thigh meat from their offspring. Livest. Sci. 164:102–108.

Cheng, L. C., Y. Yu, Q. S. Zhang, A. Szabo, H. Q. Wang, and X. F. Huang. 2015. Arachidonic acid impairs hypothalamic leptin signaling and hepatic energy homeostasis in mice. Mol. Cell. Endocrinol. 412:12–18.

Collison, L. W., C. J. Workman, T. T. Kuo, K. Boyd, Y. Wang, K. M. Vignali, R. Cross, D. Sehy, R. S. Blumberg, and D. A. Vignali. 2007. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature 450:566–569.

Costantini, L., R. Molinari, B. Farinon, and N. Merendino. 2017. Impact of omega-3 fatty acids on the gut microbiota. Int. J. Mol. Sci. 18:2645–2662.

Denbow, D. M., S. Meade, A. Robertson, J. P. McMurtry, M. Richards, and C. Ashwell. 2000. Leptin-induced decrease in food intake in chickens. Physiol. Behav. 69:359–362.

Ding, J. M., R. H. Dai, L. Y. Yang, C. He, K. Xu, S. Y. Liu, W. J. Zhao, L. Xiao, L. X. Luo, Y. Zhang, and H. Meng. 2017. Inheritance and establishment of gut microbiota in chickens. Front Microbiol 8:1967.

Ding, P., H. C. Liu, Y. Y. Tong, X. He, X. Yin, Y. L. Yin, H. H. Zhang, and Z. H. Song. 2022. Developmental change of yolk microbiota and its role on early colonization of intestinal microbiota in chicken embryo. Animals 12:16.

Donohoe, D. R., N. Garge, X. Zhang, W. Sun, T. M. O'Connell, M. K. Bunger, and S. J. Bultman. 2011. The microbiome andbutyrate regulate energy metabolism and autophagy in themammalian colon. Cell Metab 13:517–526.

Dridi, S., Q. Swennen, E. Decuypere, and J. Buyse. 2005. Mode of leptin action in chicken hypothalamus. Brain Res 1047:214–223.

Druyan, S. 2010. The effects of genetic line (broilers vs layers) on embryo development. Poult. Sci. 89:1457–1467.

Feng, R. B., L. J. Ma, M. Wang, C. H. Liu, R. J. Yang, H. X. Su, Y. Yang, and J. B. Wan. 2020. Oxidation of fish oil exacerbates alcoholic liver disease by enhancing intestinal dysbiosis in mice. Commun. Biol. 3:481.

He, L., T. He, S. Farrar, L. B. Ji, T. Y. Liu, and X. Ma. 2017. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. Cell. Physiol. Biochem. 44:532–553.

Huttenhower, C., D. Gevers, R. Knight, S. Abubucker, and O. White. 2012. The human microbiome project (hmp) consortium. structure, function and diversity of the healthy human microbiome. Nature 486:207–214.

Ibrahim, D., R. El-Sayed, S. I. Khater, E. N. Said, and S. A. M. El-Mandrawy. 2018. Changing dietary n-6: n-3 ratio using different oil sources affects performance, behavior, cytokines mRNA expression and meat fatty acid profile of broiler chickens. Anim. Nutr. 4:44–51.

Jha, R., A. K. Singh, S. Yadav, J. F. D. Berrocoso, and B. Mishra. 2019. Early nutrition programming (*in ovo* and Post-hatch Feeding) as a strategy to modulate gut health of poultry. Front. Vet. Sci. 6:82–91.

Lee, S., T. M. La, H. J. Lee, I. S. Choi, C. S. Song, S. Y. Park, J. B. Lee, and S. W. Lee. 2019. Characterization of microbial communities in the chicken oviduct and the origin of chicken embryo gut microbiota. Sci. Rep. 9:6838.

Li, S. Z., L. H. Zhi, Y. L. Liu, J. Shen, L. Liu, J. H. Yao, and X. Y. Yang. 2016. Effect of in ovo feeding of folic acid on the folate metabolism, immune function and epigenetic modification of immune effector molecules of broiler. Br. J. Nutr. 115:411–421.

Long, S., S. J. Liu, D. Wu, S. Mahfuz, and X. S. Piao. 2020. Effects of dietary fatty acids from different sources on growth performance, meat quality, muscle fatty acid deposition, and antioxidant capacity in broilers. Animals (Basel) 10:508–520.

Martindale, R. G., D. Berlana, J. I. Boullata, W. Cai, P. C. Calder, G. H. Deshpande, D. Evans, A. Garcia-de-Lorenzo, O. J. Goulet, A. Li, K. Mayer, M. S. Mundi, M. Muscaritoli, L. Pradelli, M. Rosenthal, J. M. Seo, D. L. Waitzberg, and S. Klek. 2020.

Summary of proceedings and expert consensus statements from the international summit "Lipids in Parenteral Nutrition". J. Parenter. Enteral. Nutr. 44:S7–S20.
Messina, M., G. Shearer, and K. Petersen. 2021. Soybean oil lowers circulating cholesterol levels and coronary heart disease risk, and has no effect on markers of inflammation and oxidation. Nutrition 89:111343.
Miles, E. A., C. E. Childs, and P. C. Calder. 2021. Long-chain polyunsaturated fatty acids (LCPUFAs) and the developing immune system: a narrative review. Nutrients 13:247–267.
Morgan, D. A., F. W. Ruscetti, and R. Gallo. 1976. Selective in vitro growth of T lymphocytes from normal human bone marrows. Science 193:1007–1008.
Mujico, J. R., G. C. Baccan, A. Gheorghe, L. E. Díaz, and A. Marcos. 2013. Changes in gut microbiota due to supplemented fatty acids in diet-induced obese mice. Br. J. Nutr. 110:711–720.
Park, J. H., J. H. Park, Y. B. Sim, S. H. Kim, and H. D. Park. 2019. Formation of a dynamic membrane altered the microbial community and metabolic flux in fermentative hydrogen production. Bioresour. Technol. 282:63–68.
Pcca, B. 2020. Eicosapentaenoic and docosahexaenoic acid derived specialised pro-resolving mediators: concentrations in humans and the effects of age, sex, disease and increased omega-3 fatty acid intake - sciencedirect. Biochimie 178:105–123.
Rees, D., E. A. Miles, T. Banerjee, S. J. Wells, C. E. Roynette, K. W. Wahle, and P. C. Calder. 2006. Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. Am. J. Clin. Nutr. 83:331–342.
Resende, M. T., C. Linder, and Z. Wiesman. 2021. Low-field nuclear magnetic resonance time domain characterization of polyunsaturated fatty acid-rich linseed and fish oil emulsions during thermal air oxidation. J. Am. Oil Chem. Soc. 98:495–508.
Ridaura, V. K., J. J. Faith, F. E. Rey, J. Cheng, A. E. Duncan, A. L. Kau, N. W. Griffin, V. Lombard, B. Henrissat, J. R. Bain, M. J. Muehlbauer, O. Ilkayeva, C. F. Semenkovich, K. Funai, D. K. Hayashi, B. J. Lyle, M. C. Martini, L. K. Ursell, J. C. Clemente, W. Van Treuren, W. A. Walters, R. Knight, C. B. Newgard, A. C. Heath, and J. I. Gordon. 2013. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 341:1241214.
Robertson, R. C., C. S. Oriach, K. Murphy, G. M. Moloney, J. F. Cryan, T. G. Dinan, R. P. Ross, and C. Stanton. 2016. Omega-3 polyunsaturated fatty acids critically regulate behaviour and gut microbiota development in adolescence and adulthood. Brain Behav. Immun. 59:21–37.
Roto, S. M., Y. M. Kwon, and S. C. Ricke. 2016. Applications of *in ovo* technique for the optimal development of the gastrointestinal tract and the potential influence on the establishment of its microbiome in poultry. Front. Vet. Sci. 3:63.
Ryan, M. P., and C. C. Adley. 2014. Ralstonia spp.: emerging global opportunistic pathogens. Eur. J. Clin. Microbiol. Infect. Dis. 33:291–304.
Schmilovitz-Weiss, H., A. Tovar, M. Halpern, J. Sulkes, M. Braun, Y. Rotman, R. Tur-Kaspa, and Z. Ben-Ari. 2006. Predictive value of serum globulin levels for the extent of hepatic fibrosis in patients with chronic hepatitis B infection. J. Viral Hepat. 13:671–677.
Serhan, C. N. 2017. Discovery of specialized pro-resolving mediators marks the dawn of resolution physiology and pharmacology. Mol. Aspects Med. 58:1–11.
Serhan, C. N., N. Chiang, and J. Dalli. 2018. New pro-resolving n-3 mediators bridge resolution of infectious inflammation to tissue regeneration. Mol. Aspects Med. 64:1–17.
Shakouri, M. D., P. A. Iji, L. L. Mikkelsen, and A. J. Cowieson. 2009. Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. J. Anim. Physiol. Anim. Nutr. 93:647–658.
Speake, B. K., A. M. Murray, and R. C. Noble. 1998. Transport and transformations of yolk lipids during development of the avian embryo. Prog. Lipid Res. 37:1–32.
Swain, S. L., A. D. Weinberg, M. English, and G. Huston. 1990. IL-4 directs the development of Th2-like helper effectors. J. Immunol. 145:3796–3806.
Turnbaugh, P. J., R. E. Ley, M. A. Mahowald, V. Magrini, E. R. Mardis, and J. I. Gordon. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027–1031.
Wang, G., S. Huang, Y. M. Wang, S. Cai, H. T. Yu, H. B. Liu, X. F. Zeng, G. L. Zhang, and S. Y. Qiao. 2019a. Bridging intestinal immunity and gut microbiota by metabolites. Cell. Mol. Life Sci. 76:3917–3937.
Wang, H., S. S. Liang, X. Y. Li, X. J. Yang, F. Y. Long, and X. Yang. 2019b. Effects of encapsulated essential oils and organic acids on laying performance, egg quality, intestinal morphology, barrier function, and microflora count of hens during the early laying period. Poult. Sci. 98:6751–6760.
Wang, Y. W., C. J. Field, and J. S. Sim. 2000. Dietary polyunsaturated fatty acids alter lymphocyte subset proportion and proliferation, serum immunoglobulin G concentration, and immune tissue development in chicks. Poult. Sci. 79:1741–1748.
Wu, X. Z., Z. G. Wen, and J. L. Hua. 2019. Effects of dietary inclusion of lactobacillus and inulin on growth performance, gut microbiota, nutrient utilization, and immune parameters in broilers. Poult. Sci. 98:4656–4663.
Yang, X., S. S. Liang, F. S. Guo, Z. Z. Ren, X. J. Yang, and F. Y. Long. 2020. Gut microbiota mediates the protective role of Lactobacillus plantarum in ameliorating deoxynivalenol-induced apoptosis and intestinal inflammation of broiler chickens. Poult. Sci. 99:2395–2406.
Yaqoob, P., H. S. Pala, M. Cortina-Borja, E. A. Newsholme, and P. C. Calder. 2000. Encapsulated fish oil enriched in alpha-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. Eur. J. Clin. Invest. 30:260–274.
Zhang, H., Y. Guo, and J. Yuan. 2005. Conjugated linoleic acid enhanced the immune function in broiler chicks. Br. J. Nutr. 94:746–752.
Zhu, Y. F., S. Z. Li, Q. Z. Sun, and X. J. Yang. 2019. Effect of in ovo feeding of vitamin C on antioxidation and immune function of broiler chickens. Animal 13:1927–1933.