Effects of Dietary Fish Oil Supplementation on Immune Function in Laying Hens: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary fish oil supplementation on humoral and cellular immune function in laying hens. A total of 450 healthy 31-week-old Hy-Line Brown laying hens were selected and randomly divided into 5 groups with 6 replicates per group and 15 hens per replicate. The control group was fed a corn-soybean meal basal diet, while the experimental groups were fed the basal diet supplemented with 0.54%, 1.08%, 2.17%, and 4.34% fish oil (docosahexaenoic acid contents of 0.68, 1.35, 2.70, and 5.40 mg/g, respectively). The experiment consisted of a 1-week pre-trial period and a 12-week formal trial period. The results showed that: 1) Dietary fish oil supplementation had no significant effect on spleen index, thymus index, or serum immunoglobulin A content in laying hens ($P>0.05$). 2) At 7 and 14 days post-immunization, serum antibody levels against avian influenza H9 in the experimental groups were significantly higher than those in the control group ($P<0.05$), with the 2.17% fish oil group showing the highest antibody level; at 28 days post-immunization, serum antibody levels against avian influenza H9 in the experimental groups tended to be higher than those in the control group ($P<0.10$). At 7 and 14 days post-immunization, serum antibody levels against Newcastle disease in the experimental groups were significantly higher than those in the control group ($P<0.05$); at 28 days post-immunization, serum antibody levels against Newcastle disease in the experimental groups showed no significant difference compared with the control group ($P>0.05$). 3) Compared with the control group, the relative expression levels of interleukin-2 and interleukin-6 genes in the spleen of laying hens in the 4.34% fish oil group were significantly decreased ($P<0.05$), while the relative expression levels of interleukin-10 and interferon- genes in the spleen of laying hens in the 1.08% and 2.17% fish oil groups were significantly increased ($P<0.05$). 4) Except for the 4.34% fish oil group, dietary fish oil supplementation had no significant effect on spleen lymphocyte stimulation index and apoptosis rate in laying hens

(P>0.05). In conclusion, dietary supplementation with 0.54%~2.17% fish oil could effectively improve immune function in laying hens, while excessive supplementation (4.34%) was not beneficial.

Full Text

Effects of Dietary Fish Oil on Immune Function of Laying Hens

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Abstract

This experiment was conducted to investigate the effects of dietary fish oil on humoral and cellular immune function in laying hens. Four hundred and fifty healthy 31-week-old Hy-Line Brown laying hens were randomly allocated into 5 groups with 6 replicates per group and 15 hens per replicate. The control group was fed a corn-soybean meal basal diet, while the experimental groups were fed the basal diet supplemented with 0.54%, 1.08%, 2.17%, and 4.34% fish oil (containing 0.68, 1.35, 2.70, and 5.40 mg/g docosahexaenoic acid, respectively). The trial lasted for 12 weeks following a 1-week adaptation period. The results showed that: 1) dietary fish oil supplementation had no significant effect on spleen index, thymus index, or serum immunoglobulin A (IgA) content in laying hens (P>0.05). 2) At 7 and 14 days post-immunization, serum avian influenza H9 antibody levels in the experimental groups were significantly higher than in the control group (P<0.05), with the 2.17% fish oil group showing the highest antibody titer. At 28 days post-immunization, the experimental groups showed a tendency for higher H9 antibody levels compared to the control (P<0.10). Similarly, at 7 and 14 days post-immunization, serum Newcastle disease antibody levels in the experimental groups were significantly elevated (P<0.05), though no significant difference was observed at 28 days (P>0.05). 3) Compared with the control, the 4.34% fish oil group exhibited significantly decreased relative expression of interleukin-2 (IL-2) and interleukin-6 (IL-6) genes in the spleen (P<0.05), while the 1.08% and 2.17% fish oil groups showed significantly increased expression of interleukin-10 (IL-10) and interferon- (IFN-) genes (P<0.05). 4) Except for the 4.34% fish oil group, dietary fish oil supplementation had no significant effect on spleen lymphocyte stimulation index or apoptosis rate (P>0.05). In conclusion, dietary supplementation with 0.54% to 2.17% fish oil effectively improved immune function in laying hens, whereas excessive levels (4.34%) provided no additional benefit.

Keywords: fish oil; docosahexaenoic acid; inflammatory cytokine; antibody

level; laying hen

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Introduction

As a nutritional modulator, -3 long-chain polyunsaturated fatty acids (PUFA) have received increasing attention for their roles in regulating inflammatory responses and immune function. These fatty acids participate in oxidative energy supply, modulate lipid mediator synthesis, regulate cytokine release and activation, and are incorporated into cell membranes where they influence membrane structure, thereby controlling inflammatory reactions and affecting the function of various immune cells [1]. Nutritional immunology, which utilizes specific nutrients to modulate immune status and enhance immune function, has gained significant attention from scholars worldwide. Immunonutrients can alter the host immune response to pathogens, and when fed at levels above normal requirements, can modify immune reactions. Populations consuming -3 PUFA-enriched diets show lower incidence of inflammatory and autoimmune diseases, demonstrating the immunomodulatory and anti-inflammatory activities of -3 PUFA [2]. Currently, most immunomodulators used in poultry are substances that exert anti-inflammatory effects in human disease models [3].

As an immunonutrient, PUFA is clinically used to treat inflammatory diseases. Upon inflammatory or immune stimulation, phospholipase A2 (PLA2) catalyzes the cleavage of phosphoglycerides, producing lysophosphatidylcholine (LPC) and eicosanoid precursors [1]. Representative eicosanoids include arachidonic acid (AA) and prostaglandins (PGE). During acute inflammatory responses, PGE2 (derived from AA) not only exerts pro-inflammatory effects but also activates the downstream nuclear factor- B (NF- B) signaling pathway, elevating levels of pro-inflammatory cytokines including tumor necrosis factor- (TNF-), interleukin-1 (IL-1), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS). When -3 PUFA serve as precursors, they produce PGE3 with weaker pro-inflammatory effects, thereby attenuating inflammatory responses and producing anti-inflammatory effects [1]. Additionally, -3 PUFA suppress inflammatory reactions by inhibiting major histocompatibility complex class II (MHC II) expression in dendritic cells, thereby impairing antigen presentation [2]. -3 PUFA have potential immunomodulatory effects on poultry immune responses [4-5]. Fish oil, a high-quality source of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), serves as a precursor for lipid mediators of inflammatory responses and possesses anti-inflammatory and immunomodulatory functions [6]. Current research on the immunological effects of -3 PUFA has primarily focused on human health, with limited studies on poultry, particularly peak-laying hens. Therefore, this experiment investigated the effects of different dietary fish oil levels on immune organ indices,

humoral immunity, and cellular immunity in peak-laying hens to provide scientific guidance on the immunomodulatory effects of -3 PUFA and evidence for the clinical relationship between nutrition and immunity.

1. Materials and Methods

1.1 Experimental Design and Diets Four hundred and fifty healthy 31-week-old Hy-Line Brown laying hens with similar body condition and laying rate were randomly allocated into 5 groups using a single-factor completely randomized design, with 6 replicates per group and 15 hens per replicate. The control group (Group 1) was fed a corn-soybean meal basal diet, while the experimental groups (Groups 2-5) were fed the basal diet supplemented with 0.54%, 1.08%, 2.17%, and 4.34% fish oil, respectively. The DHA content in these diets was designed to be 0.67, 1.35, 2.70, and 5.40 mg/g (actual measured values were 0.76, 1.13, 2.73, and 4.55 mg/g). Fish oil was purchased from Foshan Damao Feed Co., Ltd., with a measured DHA content of 125 mg/g. All diets were formulated to be isonitrogenous and isoenergetic according to NY/T 33—2004 standards. Diet composition and nutrient levels are presented in Table 1 , and fatty acid composition is shown in Table 2 .

1.2 Animal Management Hens were housed in three-tier battery cages with 3 hens per cage. Random numbering was used to assign group locations to avoid environmental and positional effects. Birds had free access to feed and water and were exposed to natural light supplemented with artificial lighting (16 h/d) at an intensity of 20 lx. The house temperature was maintained at (20±2)°C with relative humidity of 50%-60%. Ventilation consisted of natural ventilation combined with longitudinal negative pressure. Manure was removed twice daily, and disinfection was performed weekly. Routine immunization was carried out. Feed was provided three times daily (08:00, 13:00, and 18:00), and eggs were collected once daily. The experiment included a 1-week pre-trial period followed by a 12-week formal trial period.

1.3 Measurement Indicators and Methods

1.3.1 Immune Organ Index Determination At the end of the 12-week trial, 2 hens with similar body weight were selected from each replicate, weighed, and euthanized by cervical venous exsanguination. The spleen and thymus were collected and weighed. Thymus index (%) = 100 × thymus wet weight / body weight; Spleen index (%) = 100 × spleen wet weight / body weight.

1.3.2 Serum Immunoglobulin A (IgA) Content Determination At the end of the 12-week trial, one hen from each replicate was selected. Fasting blood samples (2-3 mL) were collected aseptically from the wing vein using procoagulant tubes, incubated in a 37°C water bath for 6-7 h, and serum was

harvested and stored at -20°C. Serum IgA content was measured using an avian IgA ELISA kit from Abcam.

1.3.3 Serum Newcastle Disease and Avian Influenza H9 Antibody Level Determination Hens from the control group (Group 1) and Groups 3-5 were intramuscularly immunized with a bivalent inactivated vaccine (Newcastle disease virus La Sota strain and avian influenza virus H9 subtype SS/94 strain). Blood samples were collected before vaccination and at 7, 14, and 28 days post-vaccination for serum preparation (as described in 1.3.2). Avian influenza H9 antibody levels were detected using the IDVET avian influenza H9 antibody competitive ELISA kit, with results expressed as S/N% (sample OD value/negative OD value × 100%). Lower S/N% values indicate higher H9 antibody levels. Newcastle disease antibody levels were measured using the IDVET Newcastle disease antibody ELISA kit.

1.3.4 Spleen Cytokine Gene Expression Determination At the end of the 12-week trial, one hen from each replicate was euthanized by cervical venous exsanguination, and approximately 10 g of spleen tissue was collected in RNA protectant solution and stored in liquid nitrogen. After thawing the RNA protectant solution at 4°C, 80 mg of tissue was placed in a 1.5 mL enzyme-free tube containing 1 mL Trizol and homogenized at 60 Hz for 60 s using a sample grinder (Shanghai Jingxin Technology) to fully lyse spleen cells. Chloroform (200 L) was added, vortexed for 30 s, incubated at 4°C for 3 min, and centrifuged at 12,000 r/min for 15 min. The aqueous phase was transferred to a new enzyme-free tube, mixed with an equal volume of isopropanol, incubated at 4°C for 30 min, and centrifuged at 10,000 r/min for 10 min. The supernatant was discarded, the pellet was washed twice with 75% ethanol, air-dried, and dissolved in 20-50 L enzyme-free water for storage at -80°C.

Total RNA concentration and purity were measured using a UV-visible spectrophotometer (BioSpec-nano, Japan). All samples had OD260/OD280 ratios between 1.8-2.0, and total RNA integrity was verified by 1% agarose gel electrophoresis (Liuyi Instrument Factory, DYY-6C). Total RNA was reverse-transcribed to cDNA using the Tiangen FastQuant RT Kit (with gDNase) according to the manufacturer' s instructions. The resulting cDNA was stored at -20°C or used directly for real-time quantitative PCR.

Real-time quantitative PCR was performed to detect target gene expression using the Tiangen SuperReal PreMix Plus (SYBR Green) kit on a real-time PCR system (Bio-Rad, USA). Based on conserved nucleotide sequences of chicken IL-2, IL-6, IL-10, and IFN- genes published in GenBank, 5 pairs of specific primers were designed and synthesized (primer sequences shown in Table 3). -actin served as the reference gene, and relative gene expression was calculated using the $2-\Delta\Delta Ct$ method, where ΔCt = Ct(target gene) - Ct(reference gene) and $\Delta\Delta Ct$ = ΔCt(treatment group) - ΔCt(control group).

1.3.5 Spleen Lymphocyte Proliferation and Apoptosis Determination
Spleen lymphocyte preparation: At the end of the 12-week trial, one hen from each replicate in the control and Groups 3-5 was selected. Spleens were aseptically collected, and approximately 10 g of tissue was placed on a 200-mesh cell strainer with 1-2 mL sterile Hank' s solution. Tissue was gently ground with a sterile glass pestle to prepare a single-cell suspension. Cells were centrifuged at 1,000 r/min for 10 min (4°C), resuspended in Hank' s solution, and centrifuged again under the same conditions. The cell pellet was suspended in 2 mL RPMI1640 medium and diluted to 2×10 cells/mL.

Apoptosis detection: The Beyotime Annexin V-FITC apoptosis detection kit was used. Spleen lymphocyte suspensions were washed twice with cold phosphate-buffered saline (PBS) and resuspended in 1× binding buffer at 1×10 cells/mL. A 100 L aliquot (approximately 1×10 cells) was transferred to a 5 mL culture tube, and 5 L FITC Annexin V and 5 L propidium iodide (PI) were added. Samples were gently vortexed and incubated at 25°C in the dark for 15 min, then 400 L of 1× binding buffer was added. Apoptosis levels were measured by flow cytometry (Guava® easyCyte, USA) within 1 h.

Proliferation detection: Lymphocyte culture was performed using 96-well plates with splenocytes at a final concentration of 2×10 cells/mL, adding 10 L per well in duplicate. The first well received 90 L RPMI1640 medium containing concanavalin A (ConA, SIGMA) at 20 g/mL, while the second well received 90 L RPMI1640 medium alone. Four blank control wells (medium only) were included. Cells were cultured at 37°C in a 5% CO incubator for 72 h. Lymphocyte proliferation was measured using the Abcam BrdU Cell Proliferation ELISA Kit. Between 48-72 h of culture, 10 L of diluted BrdU labeling solution was added per well and incubated for 12-24 h. Proliferation was assessed following the kit protocol, with OD values measured at 450/540 nm dual wavelengths.

1.4 Statistical Analysis Experimental data were analyzed using one-way ANOVA in SPSS 19.0 software. Duncan' s multiple range test was used for post-hoc comparisons and correlation analysis. Significance was declared at $P<0.05$, and trends were noted at $P<0.10$. Results are expressed as means and standard errors.

2. Results and Analysis

Production performance results from this experiment (unpublished data) showed that different fish oil levels had no significant effect on laying rate, average egg weight, egg mass, or average daily feed intake throughout the trial ($P>0.05$), though the 4.34% fish oil group (Group 5) had a significantly higher feed-to-egg ratio than the control ($P<0.05$).

2.1 Effects of Dietary Fish Oil on Immune Organ Indices and Humoral Immunity As shown in Table 4 , dietary fish oil supplementation had no sig-

nificant effect on spleen index, thymus index, or serum IgA content in laying hens (P>0.05), though the 4.34% fish oil group showed the highest serum IgA content. Results for serum avian influenza H9 antibody levels at different time points post-immunization (Figure 1 [Figure 1: see original paper]) indicated that at 7 and 14 days post-immunization, the experimental groups had significantly lower S/N% values (P<0.05), indicating significantly higher H9 antibody levels than the control, with the 2.17% fish oil group showing the highest antibody titer (though no significant differences existed among experimental groups, P>0.05). At 28 days post-immunization, the experimental groups showed a tendency for lower S/N% values (P<0.10), indicating a trend toward higher H9 antibody levels, with the 4.34% fish oil group having the highest titer. As time post-immunization increased, S/N% values decreased in all groups, indicating gradually increasing H9 antibody levels.

Results for serum Newcastle disease antibody levels at different time points (Figure 2 [Figure 2: see original paper]) showed that at 7 days post-immunization, the experimental groups had significantly higher antibody levels than the control (P<0.05), with the 1.08% fish oil group showing the highest titer. At 14 days post-immunization, experimental groups maintained significantly higher levels (P<0.05) with no significant differences among them (P>0.05). By 28 days post-immunization, no significant differences were observed between experimental and control groups (P>0.05), though the 1.08% fish oil group maintained the highest antibody level. All groups showed increasing Newcastle disease antibody levels over time post-immunization.

2.2 Effects of Dietary Fish Oil on Spleen Cytokine Gene Expression The effects of dietary fish oil on spleen cytokine gene expression are shown in Figure 3 [Figure 3: see original paper]. The 0.54%, 1.08%, and 2.17% fish oil groups showed no significant differences in relative expression of IL-2 and IL-6 genes compared with the control (P>0.05), while the 4.34% fish oil group exhibited significantly lower expression of both genes (P<0.05). Compared with the control, the 1.08% and 2.17% fish oil groups showed significantly increased IL-10 gene expression (P<0.01), whereas the 0.54% and 4.34% groups showed no significant differences (P>0.05). For IFN- gene expression, the 0.54% and 2.17% fish oil groups showed significant increases (P<0.05), the 1.08% group showed a highly significant increase (P<0.01), and the 4.34% group showed a significant decrease (P>0.05).

2.3 Effects of Dietary Fish Oil on Spleen Lymphocyte Stimulation Index and Apoptosis Rate The effects of dietary fish oil on spleen lymphocyte stimulation index and apoptosis rate are presented in Figure 4 [Figure 4: see original paper]. Compared with the control, the 1.08% and 2.17% fish oil groups showed no significant difference in ConA-stimulated lymphocyte transformation (stimulation index) (P>0.05), while the 4.34% fish oil group showed a significant reduction (P<0.05). No significant differences in spleen lymphocyte apoptosis rate were observed among any groups (P>0.05).

3. Discussion

3.1 Effects of Dietary Fish Oil on Immune Organ Indices and Humoral Immunity In this experiment, dietary fish oil at different levels had no significant effect on immune organ indices in laying hens. However, some studies have found that supplementing broiler diets with 3% fish oil (DHA accounting for 21.9% of total fatty acids) significantly affected thymus weight but not spleen weight [7], which partially contradicts our findings. These discrepancies may be attributed to differences in fatty acid composition of fish oil, poultry breed, or age. IgA is the primary immunoglobulin in external secretions, existing as both serum IgA and secretory IgA (sIgA). Serum IgA is mainly produced by plasma cells in mesenteric lymphoid tissues and possesses antibacterial and antiviral properties. Dietary fish oil has demonstrated significant clinical, immunological, and biochemical effects in many animal disease models, including increased organ transplant survival rates in humans and reduced proteinuria in mice with autoimmune glomerulonephritis (IgA nephropathy). This may occur through competitive inhibition of cyclooxygenase and lipoxygenase activity on AA by -3 PUFA, reducing leukotriene B4 (LTB4) production by neutrophils and monocytes and replacing it with non-inflammatory PGE3 and leukotriene B5 (LTB5) [8], thereby decreasing IgA deposition in glomerular mesangium. During inflammatory states, -3 PUFA in fish oil reduces IgA production and alleviates inflammatory diseases. However, research on the effects of fish oil on peripheral blood IgA levels in healthy subjects remains limited. Our study found no significant effect of dietary fish oil on serum IgA content in laying hens, possibly because the hens were in a healthy physiological state with normal eicosanoid secretion. Further research is needed to clarify the effects of fish oil on serum IgA levels in healthy poultry.

Humoral immunity encompasses peripheral blood immunoglobulin content and antibody-mediated specific and non-specific immunity. Avian influenza is a syndrome of avian infections caused by type A influenza viruses that severely impacts poultry production. Our results showed that H9 antibody levels in experimental groups were significantly higher than the control at 7 and 14 days post-immunization, with this trend continuing through 28 days. Previous studies using hemagglutination inhibition assays found that dietary supplementation with 3% fish oil significantly increased Newcastle disease antibody levels [7]. He et al. [9] reported that supplementing poultry diets with 4.5% fish oil significantly increased Newcastle disease antibody levels in both normal and cyclophosphamide-immunocompromised chickens at 14 days after primary immunization and 9 days after secondary immunization, indicating that -3 PUFA-rich fish oil can enhance humoral immunity under both normal and immunosuppressive conditions. These findings align with Guo et al. [10], though other reports show no improvement in humoral immunity with fish oil supplementation [11], possibly due to differences in dietary basal composition, fatty acid levels, or antigen types. In our experiment, hens showed no adverse reactions to the bivalent vaccine, and dietary -3 PUFA supplementation improved humoral

immunity and increased antibody titers.

3.2 Effects of Dietary Fish Oil on Spleen Cytokine Gene Expression
Cytokines are small peptide or protein molecules with immunomodulatory functions produced by immune cells during inflammatory and immune responses. Cytokine production is regulated by eicosanoids, and dietary fatty acids affect eicosanoid synthesis, thus influencing cytokine production. Studies have shown that DHA can modulate immune cell proliferation and natural killer cell activation, thereby affecting cytokine production [1]. T cells can differentiate into functionally distinct Th1 and Th2 subsets, with IL-2 and TNF mainly secreted by Th1 cells, while interleukin-4 (IL-4), interleukin-5 (IL-5), and IL-10 are produced by Th2 cells [12]. Cytokines including IL-2, IL-6, and TNF are primary mediators of inflammatory responses [13].

Friedman et al. [14] reported that either excessive or insufficient -3 PUFA can inhibit IL-2 secretion in vivo and in vitro. Compared with low -3 PUFA controls, dietary -3 PUFA supplementation reduced IL-1 , IL-2, IL-6, and TNF- levels in human peripheral blood mononuclear cells [2]. Some in vitro studies have shown that high-level -3 PUFA decreases expression of IL-1 and IL-2 genes in animal cells [15]. Our experiment demonstrated that IL-2 gene expression decreased with increasing fish oil supplementation, possibly because increased dietary -3 PUFA content reduced LTB4 production. LTB4 derived from -6 PUFA can increase vascular permeability and blood flow while regulating production of pro-inflammatory mediators TNF- , IL-2, IL-6, and IL-1 [13]. Additionally, weakened expression of Toll-like receptors 2 and 4 (TLR2, TLR4) and activation molecules may reduce intracellular signaling protein expression in peripheral blood mononuclear cells, leading to decreased IL-2 and TNF- expression with fish oil supplementation [16]. Mayer et al. [17] reported that fish oil-derived -3 PUFA significantly inhibited IL-6 and IL-8 release from monocytes stimulated with endotoxin, and although surface adhesion factor expression was unchanged, transmembrane transport and adhesion to endothelial cells were suppressed. Our results showed that IL-6 expression also decreased with increasing dietary fish oil levels. Inhibition of NF- B activation by -3 PUFA represents an important mechanism for suppressing inflammatory factor production and modulating immune cell responses [18]. Studies have shown that daily supplementation with 1.1-5.0 g EPA+DHA for several weeks in humans reduced peripheral blood mononuclear cell proliferation and decreased expression of IL-2, IL-1, IL-6, and TNF- [19].

IL-10 is an anti-inflammatory cytokine; following trauma, the body immediately produces anti-inflammatory factors including IL-10 and interleukin-13 (IL-13) to counteract primary pro-inflammatory responses [19]. After 2 months of consuming an appropriate ratio of DHA and EPA, optimal -3 PUFA levels can activate human leukocytes and promote IFN- secretion [20]. Mice fed fish oil-enriched diets showed increased IFN- levels [21], and Sijben et al. [22] reported that dietary fish oil supplementation increased cytokine production such as IFN- . Supple-

menting diets with 3% fish oil (DHA accounting for 21.9% of total fatty acids) significantly increased spleen IFN- gene expression, possibly due to enhanced innate immune cell activity (e.g., Th2 cells) and reduced eicosanoid production [7]. In vitro studies have also shown that fish oil promotes IFN- secretion by reducing PGE2 production [22]. Trauma patients showed significantly increased peripheral blood IFN- levels at 3 and 6 days after fish oil supplementation, indicating that -3 PUFA supplementation can significantly reduce post-traumatic inflammatory responses, promote recovery of cellular immune function, and prevent excessive inflammatory reactions [19].

The reduction in cytokine gene expression at high fish oil doses may be due to increased unsaturated fatty acid content in immune cell membrane phospholipids, leading to enhanced lipid peroxidation and membrane damage that affects immune cell function. Our results indicate that under normal physiological conditions without viral or other inducers, cytokine expression patterns reflect normal immune response status, and dietary fish oil supplementation is beneficial for enhancing innate and adaptive immune responses, though the effects depend on the immune response state.

3.3 Effects of Dietary Fish Oil on Spleen Lymphocyte Stimulation Index and Apoptosis Rate Our results showed that except for the 4.34% fish oil group, dietary fish oil supplementation had no significant effect on spleen lymphocyte proliferation or apoptosis. Pompos et al. [23] reported that lymphocyte proliferation under ConA stimulation was unaffected by -3 PUFA addition in vitro. Calder et al. [24] found that -3 PUFA-rich fish oil reduced ConA-stimulated lymphocyte division in mice and decreased production of IL and TNF necessary for lymphocyte development. Dietary supplementation with 7% fish oil inhibited lymphocyte proliferation [11], possibly because IL-2 produced by T cells induces T cell differentiation, and excessive -3 PUFA reduced IL-2 expression, thereby affecting T lymphocyte proliferation [25]. One study found that low concentrations (50 nmol/L) of AA, DHA, and EPA had no effect on umbilical cord blood mononuclear cell apoptosis in vitro, while medium concentrations (100 nmol/L) of DHA significantly increased monocyte apoptosis [26]. Previous research has shown that EPA+DHA supplementation at 2.4-9.6 g/d reduced neutrophil and monocyte activity, decreased expression of IL-6, IL-2, and IFN- by reducing monocyte numbers [27], and also diminished lymphocyte proliferation [28]. Kew et al. [29] provided 0.77 or 1.7 g/d EPA+DHA to 150 healthy volunteers for 6 months and found that different EPA+DHA intake levels did not alter ConA-stimulated peripheral blood lymphocyte proliferation, suggesting that appropriate -3 PUFA intake does not affect immune cell activity.

4. Conclusion

1. Dietary fish oil supplementation did not significantly affect immune organ indices or serum IgA levels in laying hens.

2. At 7 and 14 days post-immunization, fish oil groups showed significantly higher avian influenza H9 and Newcastle disease antibody levels than the control group, though no significant differences were observed among groups at 28 days.

3. Dietary supplementation with 4.34% fish oil significantly decreased spleen IL-2 and IL-6 gene expression, while 1.08% and 2.17% fish oil significantly increased spleen IL-10 and IFN- gene expression.

4. Dietary supplementation with 4.34% fish oil significantly reduced the spleen lymphocyte stimulation index, while other treatment levels showed no significant effects on lymphocyte stimulation index or apoptosis rate.

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Note: Figure translations are in progress. See original paper for figures.