Effects of Dietary Polyunsaturated Fatty Acids on Antibody Production and Lymphocyte Proliferation of Laying Hens*

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ABSTRACT : The purpose of present study was to assess the effect of polyunsaturated fatty acids (PUFA) on the immune responses of laying hens. Three hundred and sixty hens at the age of 60 weeks were randomly assigned to ten diets, which contained no oil (CK), 1%, 3%, 5% fish oil (FO); 2%, 4%, 6% linseed oil (LO) and 2%, 4%, 6% corn oil (CO). After 5 weeks of feeding experimental diets, humoral and cellular immune responses were assayed. Laying hens were injected with Sheep Red Blood Cell (SRBC) and Bovine Serum Albumin (BSA) and antibody titers, which were measured on d6, d10, d14 after primary challenge and on d5, d9, d13 after secondary challenge. Concanavalin (ConA) and lipopolysaccharide (LPS) -stimulated proliferation of peripheral blood and spleen lymphocytes were assessed by [^{3}H] thymidine incorporation at the week age of 5 and 10, respectively. The results showed that antibody titers in FO-fed and LO-fed laying hens were higher than that in laying hens fed CO. The proliferation response to ConA was lower in laying hens that fed oils rich in n-3 fatty acids than that in laying hens fed CO. Higher level n-3 fatty acids can improve immune functions of laying hens. In conclusion, dietary fat source and level had a significant impact on immune responses of laying hens. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 9 : 1320-1325)***

Key Words : Laying Hens, Dietary Polyunsaturated Fatty Acids, Humoral Immunity, Cell-mediated Immunity

INTRODUCTION

Apart from their role in supplying calories, dietary fats are recognized as an important modulator of the immune response. Both cell-mediated immunity (CMI) and humoral immunity were influenced by the amount and type of fat in the diet (Johnston, 1985; Fritsche et al., 1991). However, the specific effects of the type and level of dietary fats on the immune system are still quite controversial. Pricket et al. (1984) reported that dietary supplementation of n-3 fatty acids derived from fish oil decreased antibody production in rat. It has also been reported that n-3 fatty acids from linseed oil or fish oil had no effect on antibody production in rabbits (Kelley et al., 1988). Yoshino and Ellis (1989) reported that the mouse serum hemagglutinin titer was significantly higher in the group fed a diet with the n-6 to n-3 fatty acid ratio of 0.25 than in the group fed a diet with 2.78 or 100 of n-6 to n-3 fatty acid ratio. Dietary n-3 PUFA has been shown to enhance antibody production and cell-mediated cytotoxicity in mice and chicks (Prickett et. al., 1982; Fritsche and Johnston, 1988, 1990). It has been reported that feeding rats or mice with high levels of n-3 PUFA will result in marked suppression of in vitro spleen, thymus, lymph node, and peripheral blood lymphocyte proliferation (Alexander and Smythe, 1988; Yaqoob et al., 1994; Yaqoob and Calder, 1995；Wang et al., 2000). Feeding rats or mice with high levels of n-3 PUFA such as linolenic acid (LNA) also suppressed lymphocyte proliferation in vitro (Marshall and Johnston, 1985; Jeffery et al., 1996). Fritsche et al. (1991) reported that n-3 PUFA, LNA or EPA, and DHA significantly suppressed in vitro spleen lymphocyte proliferation in chicken. Little is known about the influence of dietary fat source and level on the immune response of laying hens.

In the present study, three fat sources were used to study the effects of immune responses on laying hens, two of which [i.e., fish oil (FO) and linseed oil (LO)] contained significant amounts of n-3 PUFA and the other one with [corn oil (CO)] contained significant amounts of n-6 PUFA. Each fat source contained three levels. Bovine Serum Albumin (BSA) and Sheep Red Blood Cell (SRBC) were selected as the antigen to influence the antibody titers of laying hens. Lymphocyte proliferation in response to two unspecific mitogens was used to assess immune cell function. Mitogens were chosen such that proliferative responses of T-and B-lymphocytes might be different. It has been reported that ConA selectively stimulates chicken T-lymphocytes (Toivanen and Toivanen, 1973). Bacterial lipopolysaccharride (LPS) selectively stimulates B-lymphocyte.

MATERIALS AND METHODS

Animals and housing

Three hundred and sixty female commercial Hisex laying hens (60-week old) were raised with 3 birds per cage. The birds had free access to feed and water. At the beginning of trial, birds were randomly assigned to the experimental treatments.

* This study was financially supported by Natural Science Foundation Council of P.R. China, with the project No. 30170689

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Received February 21, 2003; Accepted April 21, 2003

Table 1. Composition and nutrient level of diets fed to laying hens

Ingredients	CK	FO1	FO3	FO5	LO2	LO4	LO6	CO2	CO4	CO6
	%									
Corn	63.36	59.69	52.32	45.57	55.63	48.49	41.97	55.63	48.49	41.97
Wheat middlings	0	3.05	7.52	10.00	6.60	10.00	12.06	6.60	10.00	12.06
Soybean meal	25.85	25.50	25.30	25.70	25.00	25.15	25.60	25.00	25.15	25.60
Zeolite meal	0	0	1.09	2.96	0	1.59	3.60	0	1.59	3.60
Fish oil [1]	0	1.00	3.00	5.00	0	0	0	0	0	0
Linseed oil	0	0	0	0	2	4	6	0	0	0
Corn oil	0	0	0	0	0	0	0	2	4	6
Limestone meal	8.80	8.80	8.80	8.80	8.80	8.80	8.80	8.80	8.80	8.80
Monocalcium phosphate	1.35	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-Methionine	0.12	0.12	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Trace mineral premix [2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix [3]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Nutrients level (calculated)										
Metabolizable energy (MJ/kg)	11.42	11.42	11.42	11.42	11.42	11.42	11.42	11.42	11.42	11.42
Crude protein (%)	16.51	16.52	16.51	16.52	16.51	16.51	16.50	16.51	16.51	16.50
Methionine (%)	0.38	0.38	0.38	0.38	0.38	0.38	0.37.	0.38	0.38	0.37
Lysine (%)	0.80	0.80	0.80	0.81	0.80	0.80	0.81	0.80	0.80	0.81
Calcium (%)	3.50	3.49	3.49	3.50	3.49	3.50	3.50	3.49	3.50	3.50
Nonphytate (%)	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34

[1] Refined fish oil from China East Sea.

[2] Mineral premix provides per kilogram of diet: Mn, 80 mg; Zn, 90 mg; Fe, 60 mg; Cu, 12 mg; Se, 0.147mg; I, 0.3 mg.

[3] Vitamin premix supplies per kilogram of diet: retinyl acetate, 8,065 IU; cholecalciferol, 1,580 IU; 25-hydroxy-vitamin D_3, 31.5 ug; dl-α-tocopheryl acetate, 15 IU; vitamin B_{12}, 16 ug; menadione, 4 mg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; niacin, 7.5 mg; choline chloride, 509 mg; folic acid, 1.62 mg; biotin, 0.27mg.

Table 2. Fatty acids composition of oils added to laying hens' diets

Fatty acids	Composition (%)[1]		
	FO	LO	CO
16:0	20.26	5.36	11.62
16:1	9.43	0.13	0.15
18:0	4.21	6.00	2.05
18:1	19.69	21.50	31.23
18:2n-6	1.83	12.01	52.06
18:3n-3	1.69	54.18	1.02
20:1	6.28	0.21	0.27
20:4n-6	0.10	0.18	0.28
20:5n-3	17.49	0.23	-
22:6n-3	18.97	0.18	0.31

[1] % fatty acids.

Experimental design

The effects of dietary PUFA on laying hens immune functions were studied. Factors were dietary fish oil (refined fish oil from China East Sea, FO), linseed oil (LO) and corn oil (CO). Three doses of FO based on weight were: 1, 3 and 5%. Three doses of LO and CO, based on weight were 2, 4, and 6%; respectively. Diets were formulated to meet or exceed the nutrient recommendations for poultry of the NRC (1994). Table 1 shows composition of experimental diets and nutrient values. The fatty acid profiles of different fat sources that used in the present study were analyzed by gas-liquid chromatography (Cherian and Sim, 1982) (Table 2).

Production performance

From week 3 to week 9 after feeding exp. diets, animal production performance was determined on percentage of laying egg, egg weight and percentage of cracked eggs.

Antibody production

Laying hens in each treatment group (n=6) were injected i.m. with 1 mL of a 7% suspension of SRBC on the 22th day and the 42th day after feeding exp. diets. Serum antibody titers against SRBC were measured on the 6th day, 10th day and 14th day after the first injection and on the 5th day, 9th day and 13th day after the second injection by active hemagglutination test (Hudson and Hay, 1976). Titers were expressed as the $\log_2$ values of the highest dilution giving a positive reaction.

Chickens were immunized with 2 mL of 0.5% BSA on the 22th day and the 42th day after feeding exp. diets. Serum total antibody (Ab) titers to BSA were determined by ELISA from 6 birds each treatment on 6, 10 and 14 d after primary sensitization, and on 5, 9 and 13 d after booster (Friedman and Sklan, 1995). Briefly, serial dilutions of antiserum were applied to Coaster ELISA dishes previously coated with BSA. Bound anti-BSA antibodies were detected by means of polyvalent, peroxidase-labeled, rabbit anti-chicken IgG antibodies. Anti-BSA antibody binding is expressed in absorbance units at 405 nm.

Table 3. Effects of dietary fat sources and levels on laying performance of hens

Dietary fat sources and levels	Egg laying percentage (%)	Egg weight (g)	Cracked egg (%)
CK	71.53±5.42	63.59±0.55	0.93±0.93
FO1	78.70±2.63	64.04±1.45	0.29±0.50
FO3	76.85±9.48	63.76±0.16	2.10±1.20
FO5	74.23±6.83	61.37±0.62	1.60±0.64
LO2	71.06±10.31	61.67±0.55	0.97±0.89
LO4	74.77±4.63	64.56±0.52	0.30±0.51
LO6	72.92±6.30	64.38±0.15	1.49±1.39
CO2	76.62±1.97	63.54±2.94	1.29±2.24
CO4	72.58±5.21	64.17±1.22	1.43±1.75
CO6	74.46±9.07	64.88±0.38	1.00±0.93

Lymphocyte proliferation assay of peripheral blood

To assess lymphocyte proliferative responses, heparinized (20 U/mL) peripheral blood was collected by wing-vein puncture at week 5 and week 10. 100 uL heparinized peripheral blood was co-cultured with 1.9 mL RPMI-1640 medium (24 mM $NaHCO_3$, 5% fetal bovine serum, 100 U/mL penicillin and 100 ug/mL streptomycin) in 5% CO_2 incubator at 40°C for 56 h. Cells were co-incubated with ConA (final concentration, 45 ug/mL) and LPS (final concentration, 25 ug/mL). All mitogens were purchased from Sigma.

During the last 8 h of the incubation, 0.2 uCi of [^{3}H]-thymidine was added to each tube. Incorporation of radiolabel was determined by liquid scintillation counting.

Lymphocyte proliferation assay of spleen

Four hens from each treatment were killed by CO_2 inhalation on week 5 and week 10 after feeding exp. diets. Spleens were removed and placed in a 10 mL of sterile-filtered ice-cold Hanks balanced salt solution without Ca^{2+} and Mg^{2+}. Single cell suspensions of splenocytes were made by equipped with an 80-mesh stainless-steel screen. Using a 10-mL syringe without a needle, cell clumps were dispersed by several gentle washings through the sieve. The read blood cell and dead cells were removed by centrifugation of spleen cell suspension over Lymphocyte Separation Medium, density from 1.077 to 1.080 at 20°C as described by the manufacturer. Cells at the interface were collected, washed twice, and then enumerated using a Coulter Counter. Cell viability was always higher than 95%, as determined by trypan blue exclusion. Cell suspensions were diluted to a final concentration of 2×10^6 cells/mL in RPMI-1640 medium. Cells were co-incubated with ConA (final concentration, 45 ug/mL) and LPS (final concentration, 25 ug/mL) in 5 mL tube (2 mL RPMI 1640 each tube) in 5% CO_2 incubator at 40°C for 56 h. All mitogens were purchased from Sigma.

During the last 8 h of the incubation, 0.2 uCi of [^{3}H]-thymidine was added to each tube. Incorporation of radiolabel was determined by liquid scintillation counting.

Thymidine incorporation data are expressed as simulation index (SI) values where:

$$SI = \frac{\text{incorporation of } [^3H] \text{ thymidine in the absence of mitogen}}{\text{incorporation of } [^3H] \text{ thymidine in the absence of mitogen}}$$

Data for each hen are represented by the mean counts per minute from three tubes.

Statistical analysis

Data were analyzed by one-way ANOVA of SPSS 10.0, and reported as means±SD. The significance of differences among different groups was evaluated by Least Significant Difference (LSD) post-hoc mutiple comparisons test.

RESULTS AND DISCUSSION

Production performance

The effects of dietary fat sources and levels on birds' production are shown in Table 3. Different sources and levels of PUFA had no influence on egg production, egg weight and the percentage of cracked eggs. These results

Table 4. Serum antibody titers from anti-SRBC of laying hens*

Treatment	Primary response			Secondary response		
	6 d	10 d	14 d	5 d	9 d	13 d
CK	2.17±0.29	2.50±0.76 ^{a}	2.33±1.04^{a}	2.25±0.29 ^{a}	2.50±0.50^{a}	2.33±0.29^{a}
FO1	2.50±0.58	3.50±0.86 ^{ab}	3.33±0.58^{ab}	2.83±0.58 ^{ab}	3.83±0.76^{ab}	3.67±0.58^{abc}
FO3	2.67±0.50	4.00±0.50 ^{ab}	3.50±0.50 ^{ab}	2.83±0.29 ^{ab}	4.33±0.76^{b}	4.00±0.50^{bc}
FO5	3.00±1.00	4.50±0.86 ^{b}	4.33±1.53 ^{b}	4.16±1.04^{c}	4.83±0.58^{b}	4.33±0.58^{c}
LO2	2.33±0.58	3.67±1.04 ^{ab}	3.33±1.16^{ab}	2.67±0.76 ^{ab}	4.00±0.50^{ab}	3.50±0.50^{abc}
LO4	2.50±0.50	3.67±0.29 ^{ab}	3.33±0.58^{ab}	3.00±1.00 ^{abc}	4.50±1.00^{b}	3.50±0.86^{abc}
LO6	2.83±0.76	3.67±1.53 ^{ab}	3.67±0.58^{ab}	3.67±0.76 $^{b\ c}$	4.83±0.76^{b}	3.83±0.29^{bc}
CO2	2.50±0.50	3.16±0.29 ^{ab}	2.83±0.76^{ab}	2.33±0.58 ^{a}	3.50±0.50^{ab}	2.67±0.58^{ab}
CO4	2.67±0.29	3.33±0.29 ^{ab}	3.00±1.00^{ab}	2.33±0.58 ^{a}	3.33±1.53^{ab}	3.17±1.26^{abc}
CO6	2.67±0.76	3.33±0.29 ^{ab}	3.17±0.76^{ab}	2.85±0.50 ^{ab}	3.50±1.33^{ab}	3.33±1.16^{abc}

Different letters in the same column indicated significantly ($p<0.05$). * Expressed as the $\log_2$ values of the highest dilution giving a positive reaction.

Table 5. Serum antibody titers from anti- BSA of laying hens

Treatment	Primary response			Secondary response		
	6 d	10 d	14 d	5 d	9 d	13 d
CK	0.189±0.027[a]	0.196±0.073[a]	0.221±0.066[a]	0.208±0.062[a]	0.235±0.082[a]	0.228±0.047[a]
FO1	0.220±0.019[ab]	0.355±0.104[bc]	0.310±0.104[bc]	0.245±0.079[ab]	0.346±0.047[ab]	0.321±0.044[ab]
FO3	0.231±0.036[b]	0.360±0.107[bc]	0.352±0.083[bc]	0.279±0.023[ab]	0.397±0.079[bc]	0.336±0.030[bc]
FO5	0.252±0.028[b]	0.387±0.071[c]	0.381±0.031[c]	0.297±0.075[b]	0.434±0.039[c]	0.400±0.081[c]
LO2	0.218±0.020[ab]	0.339±0.055[bc]	0.338±0.049[bc]	0.248±0.043[ab]	0.345±0.035[ab]	0.300±0.042[ab]
LO4	0.219±0.033[ab]	0.337±0.070[bc]	0.340±0.053[bc]	0.272±0.052[ab]	0.352±0.048[ab]	0.320±0.047[ab]
LO6	0.245±0.015[b]	0.382±0.054[c]	0.360±0.046[c]	0.290±0.039[b]	0.399±0.052[bc]	0.350±0.069[bc]
CO2	0.217±0.026[ab]	0.269±0.061[ab]	0.241±0.076[ab]	0.246±0.030[ab]	0.327±0.035[ab]	0.324±0.068[ab]
CO4	0.215±0.024[ab]	0.287±0.054[abc]	0.321±0.107[bc]	0.266±0.042[ab]	0.325±0.056[ab]	0.301±0.045[ab]
CO6	0.216±0.023[ab]	0.310±0.068[bc]	0.277±0.121[abc]	0.270±0.071[ab]	0.334±0.075[ab]	0.325±0.084[ab]

Different letters in the same column indicated significantly ($p<0.05$). Data expressed as OD values at wavelength of 450 nm.

are consistent with those of Friedman and Sklan (1995) and Mutia (1999).

Antibody production

The effects of dietary fat sources and levels on the antibody titers of primary and secondary challenge of laying hens are shown in Table 4 and Table 5. Laying hens' antibody titers against SRBC and BSA increased as FO and LO level increased after first and second challenge. Antibody titers in 5% FO-fed chickens at 10 d and 14 d were significantly higher than those in chickens fed CK diets, but only slightly higher than low level FO-fed and CO-fed diets. Birds challenged a second time had higher total anti-SRBC titers and anti-BSA titers as compared with those receiving only first challenge. The highest antibody titers against SRBC and BSA appeared at 10 d and 9 d after primary and secondary challenge, respectively. Enhancement of antibody production by n-3 PUFA had been observed previously in mice and broiler chicks (Prickett, et al., 1982; Fritsche and Cassity, 1991; Friedman and Sklan, 1995, Parmentier et al., 1997; Sijben et al., 2001). Contrary to these observations, Phetteplace et al. (1989) reported no difference in antibody response to SRBC when chickens were fed soybean oil as compared with those fed FO. Furthermore, Kelley et al. (1988) reported no difference in antibody response by rabbits fed hydrogenated soybean oil, safflower oil or FO. Friedman and Sklan (1995) and Sijben et al. (2001) reported that Ab response decreased by feeding high levels of n-6 PUFA. These apparent discrepant observations may be the results of different antigens that the antibody (Ab) responses were directed against, and different levels were defined as high and low in n-3 and n-6 PUFAs. Because the metabolism of n-3 and n-6 PUFAs are intertwined, the measured effect of varying one PUFA might depend on the level of other PUFA and thus, on the interaction of n-3 and n-6 (Sijben et al., 2001).

It was hypothesized that dietary n-3 PUFA could enhance immune responses and diseases resistance in poultry by reducing eicosanoid production, particularly prostaglandin E_2. Diets-induced alterations in immune responses might be determined by assessing changes in the proportions of different lymphocyte subsets.

Lymphocyte proliferation of peripheral blood and splenocytes

Shown in Table 6 and Table 7 are the lymphocyte

Table 6. Effects of different dietary sources and levels of PUFA on lymphocyte proliferation of heparinized peripheral blood of laying hens on 5 w and 10 w

Treatment	Week 5		Week 10	
	ConA (SI)	LPS(SI)	ConA (SI)	LPS (SI)
CK	1.77±0.77[c]	1.23±0.29[c]	1.96±0.83[c]	1.32±0.24[d]
FO1	0.97±0.39[ab]	0.79±0.16[b]	1.19±0.21[abc]	0.95±0.09[c]
FO3	0.96±0.12[ab]	0.67±0.16[ab]	1.02±0.24[ab]	0.81±0.03[bc]
FO5	0.39±0.08[a]	0.36±0.09[a]	0.54±0.06[a]	0.45±0.13[a]
LO2	1.08±0.43[ab]	0.92±0.15[bc]	1.23±0.25[bc]	0.98±0.17[c]
LO4	1.04±0.43[ab]	0.76±0.13[b]	1.10±0.10[ab]	0.84±0.10[bc]
LO6	0.81±0.38[ab]	0.57±0.12[ab]	0.83±0.15[ab]	0.64±0.10[ab]
CO2	1.22±0.64[c]	0.91±0.12[bc]	1.27±0.42[bc]	1.08±0.19[cd]
CO4	0.99±0.33[ab]	0.88±0.31[bc]	1.13±0.21[abc]	0.97±0.14[c]
CO6	1.01±0.45[ab]	0.89±0.23[bc]	1.08±0.23[ab]	1.00±0.18[c]

Different letters in the same column indicated significantly ($p<0.05$).

Table 7. Effects of different dietary sources and levels of PUFA on splenocytes proliferation of laying hens on 5 w and 10 w

Treatment	Week 5		Week 10	
	ConA (SI)	LPS (SI)	ConA (SI)	LPS (SI)
CK	1.83±0.11[d]	1.35±0.05[d]	1.99±0.19[d]	1.63±0.22[d]
FO1	1.36±0.12[bc]	1.06±0.09[bc]	1.37±0.13[bc]	1.36±0.19[bcd]
FO	0.97±0.15[a]	0.91±0.12[ab]	1.21±0.17[ab]	1.11±0.15[ab]
FO5	0.93±0.07[a]	0.82±0.05[a]	0.95±0.09[a]	0.83±0.07[a]
LO2	1.38±0.13[bc]	1.21±0.08[cd]	1.48±0.27[bc]	1.32±0.06[bcd]
LO4	1.18±0.14[ab]	1.12±0.17[c]	1.23±0.11[ab]	1.17±0.17[abc]
LO6	0.96±0.12[a]	0.86±0.13[a]	0.96±0.05[a]	0.89±0.13[a]
CO2	1.56±0.09[cd]	1.25±0.08[cd]	1.67±0.21[cd]	1.49±0.29[cd]
CO4	1.37±0.14[bc]	1.06±0.13[bc]	1.63±0.16[c]	1.47±0.23[bcd]
CO6	1.35±0.38[bc]	1.09±0.07[bc]	1.36±0.34[bc]	1.35±0.28[bcd]

Different letters in the same column indicated significantly ($p<0.05$).

proliferation of laying hens fed the different fat sources and levels of PUFA. The present results showed that proliferative responses tended to be depressed by the n-3 rich oils, LO and FO. Proliferation in response to LPS was significantly lower in 5 % FO-fed laying hens as compared with the response from other fat sources. These results support the observation of others that dietary fat source can influence the proliferative response of lymphocytes to nonspecific mitogens (Kollmogen et al., 1979; Marshall and Johnson, 1985; Kelley et al., 1988). Many studies demonstrated that n-3 PUFA from linseed oil (rich in LNA) and fish oil (rich in EPA and DHA) can suppress lymphocyte proliferation in response to mitogen stimulation (Mashall and Johnson, 1985; Meydani et al., 1993). The EPA and DHA had a stronger suppressive effect on lymphocyte proliferation in response to Con A than LNA. This result is similar to the result from most studies with mammals (Das, 1994).

Dietary n-3 PUFA levels also influenced lymphocyte proliferation of laying hens, as the dietary n-3 PUFA level increased, the lymphocyte proliferation of laying hens were depressed more. But n-6 PUFA level seemed to have no effect on lymphocyte proliferation of laying hens. These suggest that n-3 PUFA levels was also a key factor to affect lymphocyte proliferation of laying hens. Due to different n-3 fatty acid components provided by linseed oil and fish oil, the intensities of suppressive effect of 1%, 3%, 5% fish oil and 2%, 4%, 6% linseed oil diet on lymphocyte proliferation were different. In addition, it is worth noting that the effect of n-3 PUFA on immune responses appear to be dose dependent. In this study, relatively higher dietary levels of oils (6%, wt/wt), even higher level (7%, wt/wt) was reporteded by Fritsche et al. (1991) in chicks. Both studies showed that the increase of dietary n-3 PUFA inhibited chick lymphocyte proliferative responses to ConA and LPS, and that LNA showed different potencies from EPA and DHA. However, Korver and Klasing (1997) reported that when moderate levels of n-3 PUFA (≤2%, wt/wt) were applied, increased dietary n-3 PUFA resulted in greater cell-mediated immunity in chickens as determined by the wattle delayed-type hypersensitivity. Delayed-type hypersensitivity is decreased vs. baseline at the higher level of fish oil, but there was no change at the low level of fish oil. It has also been reported that the immunosuppressive effect of dietary n-3 PUFA is relative to physiological status.

The present observations with LPS are consistent with those of Hovi et al. (1978) and Vainio and Ratcliffe (1984). This poor proliferative response to LPS may relate to the laying hen's relatively insensitivity to in vivo effects of bacterial endotoxins (Alder and DaMassa, 1978).

The lymphocyte proliferation of blood and spleen had a similar influence when different fat sources and levels PUFA added to laying hens' diets. These suggest that these factors can reflect the immune function of birds.

IMPLICATION

The results of the current research showed that the source and level of dietary PUFA could alter the immune response of laying hens. Feeding laying hens diets containing high n-3 PUFA significantly enhances antibody production. Furthermore, the source of PUFA in the diet can influence lymphocyte proliferation in response to unspecific mitogens.

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