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Maternal Dietary N-3 Fatty Acids Alter the Spleen Fatty Acid Composition and Bovine Serum Albumin-Induced Wing Web Swelling in Broilers

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ABSTRACT Effects of maternal dietary polyunsaturated fatty acids (PUFA) on the spleen fatty acid composition and BSA-induced wing web swellings were investigated in broilers. One hundred twenty broiler breeder hens 26 wk of age were randomly assigned to diets containing mainly wheat, corn, soy meal, barley, oat and 5% (wt/wt) added sunflower oil, fish oil, or a mix of sunflower and fish oils (1:1). After 2 wk on the experimental diets, birds were inseminated, eggs were collected and incubated. Progeny chicks were then fed identical diets for 6 wk. The maternal dietary oils affected ($P < 0.05$) n-6 and n-3 PUFA in the spleens of hatching chicks. After 2 wk, n-6 PUFA did not differ among the groups; n-3 PUFA, docosapentaenoic, and docosahexaenoic (DHA) acids were higher ($P < 0.05$) in the spleens of broilers from hens fed 2.5 or 5% fish oil. After 4 wk, broilers from hens fed 5% fish oil still had higher levels of DHA ($P < 0.05$) in their spleens than those from hens fed 5% sunflower oil. The BSA-induced wing web swelling response was suppressed ($P < 0.05$) by n-3 PUFA in breeder hens. Broilers from hens fed high levels of n-3 PUFA had lower ($P < 0.05$) wing web swelling reactions to BSA at 2 wk (2.5% fish oil) and 4 wk (2.5 and 5% fish oil). In conclusion, n-3 PUFA in breeder hen diets suppressed the BSA-induced wing web swellings of the hens, increased the spleen n-3 fatty acids (especially DHA), and decreased BSA-induced wing web swellings of progeny up to 4 wk of age.

(*Key words*: maternal dietary n-3 fatty acid, spleen fatty acid composition, bovine serum albumin-induced wing web swelling, broiler)

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INTRODUCTION

The immunomodulatory functions of dietary polyunsaturated fatty acids (PUFA) have been widely studied in mammals; research on poultry is limited. However, an understanding of the effects and mechanisms by which nutrition influences the immune system is necessary to appreciate the complex interactions between diet and infectious diseases and to aid in maintaining chicken health and profitability (Klasing, 1998). It has been reported that feeding high levels of n-3 PUFA (5 to 7% fish oil in the diet, wt/wt) suppresses the cell-mediated immune response, as measured by in vitro lymphocyte proliferation in chicks (Fritsche et al., 1991) and in laying hens (Wang et al., 2000a,b). In contrast, another study has shown that inclusion of low levels of n-3 PUFA (≤2% fish oil in the diet, wt/wt) in broiler chick diets improved or did not change indices of the cell-mediated immune response (Korver and Klasing, 1997). These results suggest that the effect of n-3 PUFA on chicken immune response may depend on dietary levels and may vary between chicken strains. In broilers, effects of different levels of n-3 PUFA on the cell-mediated immune response need to be elucidated.

In addition, the developmental events important for immunocompetence in chicks are initiated in the pre- and early posthatch (the first 2 wk after hatching) periods (Gobel, 1996; Ratcliff et al., 1996). The early posthatch period sees a rapid increase in leukocyte populations, seeding of lymphoid organs, and formation of unique clones of lymphocytes that will mediate immunity later in life (Klasing, 1998). Therefore, the supply of fatty acids during the embryonic and early posthatch periods may impact the development and function of the immune system. In laying chickens, the immune responses of progeny can be affected by maternal and neonatal dietary n-3 PUFA (Wang et al., 2000a). The influence of maternal n-3 PUFA alone on the

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Abbreviation Key: AA = arachidonic acid; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; FO = fish oil; PUFA = polyunsaturated fatty acid; SO = sunflower oil.

TABLE 1. Composition of fatty acids in the broiler breeder diets

Fatty acids[2]	Diet[1] (% of total fatty acids)		
	5% SO	2.5% SO + 2.5% FO	5% FO
C16:0	10.11	15.68	20.05
C18:0	3.65	3.26	2.88
C16:1	0.18	4.21	8.92
C18:1	16.89	15.59	14.39
C18:2n-6	64.99	41.48	17.79
C20:4n-6		0.31	0.59
C18:3n-3	1.73	2.25	2.52
C20:5n-3		3.92	8.59
C22:5n-3		0.68	1.48
C22:6n-3		3.06	6.55
SAFA	14.81	23.07	30.80
MUFA	17.35	20.91	25.54
PUFA	66.77	52.01	38.23
n-6 PUFA	65.02	42.03	18.91
n-3 PUFA	1.75	9.98	19.31
n-6/n-3	37.12	4.21	0.98

[1]SO = sunflower oil; FO = fish oil.

[2]SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-6/n-3 = the ratio of n-6 to n-3 PUFA.

TABLE 2. Fatty acid composition of broiler starter and grower diets

Fatty acids[1]	% of total fatty acids	
	Broiler starter	Broiler grower
C16:0	11.67	9.32
C18:0	3.50	2.32
C16:1	0.69	0.31
C18:1	43.30	50.15
C20:1	0.79	1.05
C18:2n-6	28.07	27.80
C18:3n-3	6.74	7.26
SAFA	16.32	12.79
MUFA	47.10	51.90
PUFA	34.93	35.19
n-6 PUFA	28.19	27.93
n-3 PUFA	6.74	7.26
n-6/n-3	4.18	3.85

[1]SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-6/n-3 = the ratio of n-6 to n-3 PUFA.

fatty acid profiles of immune organs, the cell-mediated immune response and the duration of action in broilers is not clearly known. The objective of the current study was to investigate the effects of breeder hen diets containing different amounts of fish oil (2.5 or 5%, wt/wt) on wing web swelling reactions to BSA in hens as well as the spleen PUFA composition and BSA-induced wing web swelling response of the progeny broilers.

MATERIALS AND METHODS

These experiments were reviewed by the University of Alberta Animal Care Committee to ensure adherence to the Canadian Council on Animal Care Guidelines.

Birds and Diets

One hundred twenty broiler breeders, 26 wk of age, were used in this study. The previous diet comprised wheat (33.7%), corn (14.3%), soy meal (13.4%), barley (15%), oats (10%), and beef tallow (2%), with a nutrient density of 15.6% protein, 2,619 kcal ME/kg. The hens were randomly assigned to one of three experimental diets with a similar composition and nutrient density as the previous diet but adjusted to include 5% (wt/wt) sunflower oil (SO), fish oil (FO), or a mixture of SO and FO (1:1). Vitamin E (27 mg/kg diet) was added to protect dietary fatty acid oxidation. Fatty acid composition of the experimental diets was analyzed by gas chromatography (Cherian and Sim, 1992) as presented in Table 1. All breeder hens were housed in individual cages with free access to feed and water. Twenty egg samples were collected on the last 2 d of each week and five eggs were analyzed for fatty acid composition. After 2 wk of feeding on the experimental diets, breeder hens were artificially inseminated once a week. Over a 6-wk period eggs were collected and stored at 13 to 15 C. There were two hatches, one from eggs during the first 4 (of 6) wk and another for the last 2 wk. Upon hatching chicks were randomly assigned to floor pens with 100 chicks per pen. There were 300 chicks from the first hatch and 200 from the second hatch, for a total of 500 chicks (five pens) per treatment. Chicks were fed identical broiler starter (1 to 3 wk) and grower (4 to 6 wk) diets. The starter diet contained 51.7% wheat/wheat short, 17.3% soy meal, 14.1% corn, 5% oats, 5% barley, and 2% beef tallow. The grower diet contained 49.4% wheat/wheat short, 16.4% corn, 12.5% oats, 10% barley, 7.4% soy meal, and 0.7% beef tallow. Broiler starter feed contained 18.2% protein, 2,783 kcal ME/kg, and 20 mg vitamin E/kg diet. The grower feed contained 15% protein, 2,711 kcal ME/kg, and 20 mg vitamin E/kg diet. Fatty acid compositions of the broiler starter and grower feeds are summarized in Table 2. There were no 20-carbon fatty acids detected in these two diets.

Spleen Collection and BSA-Induced Wing Web Swelling Measurement

On Days 0, 14, 28, and 42, spleen samples (one per pen, five per treatment) were manually harvested and stored at −20 C for fatty acid analysis. The wing web swelling reactions to BSA were measured in breeder hens after collection of fertilized eggs and their progeny in broilers at 2 and 4 wk of age. One-fifth milliliter of BSA solution (1 mg/mL in PBS) was injected subcutaneously into two sites of the left wing web. As a control vehicle, 0.2 mL of PBS was injected into two sites of the right wing web. The thickness of each injection site was measured using a pressure sensitive caliper before injection and 24 h postinjection. The wing web swelling reactions to BSA were presented by the swelling index, which was calculated as [swelling index = (thickness of left wing web following BSA injection – initial thickness of left wing web) – (thickness of right wing web following PBS injection – initial thickness of right wing web)].

TABLE 3. Fatty acid composition of egg yolk after 2 wk of feeding the experimental diets

Fatty acids[2]	Diet[1] (% of total fatty acids) 5% SO	2.5% SO + 2.5% FO	5% FO	$P <$
C16:0	26.88 ± 0.05[b]	26.88 ± 0.32[b]	29.32 ± 0.02[a]	0.0428
C18:0	9.34 ± 0.03[a]	8.28 ± 0.05[b]	8.28 ± 0.01[b]	0.0056
C16:1	2.85 ± 0.10[c]	3.84 ± 0.05[b]	5.44 ± 0.08[a]	0.0010
C18:1	34.45 ± 0.09[b]	36.73 ± 0.32[a]	37.85 ± 0.16[a]	0.0240
C18:2n-6	21.28 ± 0.05[a]	15.02 ± 0.02[b]	6.71 ± 0.02[c]	0.0001
C20:4n-6	2.51 ± 0.04[a]	1.01 ± 0.01[b]	0.61 ± 0.02[c]	0.0011
C18:3n-3	0.24 ± 0.00[c]	0.41 ± 0.01[b]	0.52 ± 0.00[a]	0.0014
C20:5n-3	0.00 ± 0.00[c]	0.38 ± 0.01[b]	1.01 ± 0.01[a]	0.0001
C22:5n-3	0.09 ± 0.00[c]	0.84 ± 0.01[b]	1.50 ± 0.00[a]	0.0002
C22:6n-3	0.52 ± 0.01[c]	4.14 ± 0.01[b]	5.48 ± 0.03[a]	0.0060
SAFA	37.02 ± 0.01[b]	36.29 ± 0.29[b]	39.14 ± 0.02[a]	0.0301
MUFA	37.48 ± 0.10[c]	41.16 ± 0.31[b]	44.08 ± 0.11[a]	0.0069
PUFA	25.23 ± 0.10[a]	21.75 ± 0.01[b]	15.03 ± 0.07[c]	0.0003
n-6 PUFA	24.37 ± 0.09[a]	16.36 ± 0.02[b]	7.54 ± 0.04[c]	0.0001
n-3 PUFA	0.86 ± 0.01[c]	5.77 ± 0.01[b]	8.50 ± 0.04[a]	0.0001
n-6/n-3	28.37 ± 0.19[a]	2.84 ± 0.01[b]	0.89 ± 0.00[c]	0.0001

[a–c]For each fatty acid, values with different superscripts are significantly different ($P < 0.05$); values are means ± SEM (n = 5).

[1]SO = sunflower oil; FO = fish oil.

[2]SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-6/n-3 = the ratio of n-6 to n-3 PUFA.

Statistical Analysis

One-way ANOVA was used to analyze the overall differences of the main effects among the three dietary treatments (there were no hatch effects). Where a significance of less than 0.05 of ANOVA was achieved, differences among treatment means were evaluated using the Student-Newman-Keuls multiple range test ($P < 0.05$; Steel and Torrie, 1980). Computations were made using the general linear models procedure of the SAS Institute (1990).

RESULTS AND DISCUSSION

Egg yolk fatty acid composition (Table 3) was significantly altered after 2 wk of feeding the experimental diets to breeder hens. The most significant change occurred in the percent composition of PUFA. The relative amount of arachidonic acid (AA; C20:4n-6) in the egg yolk was low (≤2.5%), and decreased with the increase in n-3 PUFA ($P = 0.001$). Linoleic acid (C18:2n-6) was the major n-6 PUFA in the egg yolk and was altered by the dietary supply ($P < 0.0001$). Although low, the amount of α-linolenic acid (C18:3n-3) in the egg yolk was influenced ($P = 0.001$) by diets. Eicosapentaenoic (EPA; C20:5n-3) and docosapentaenoic acids (DPA; C22:5n-3) in the yolk constituted ≤1 and ≤1.5%, respectively. The predominant n-3 fatty acid in the egg yolk was DHA (C22:6n-3). EPA, DPA, and DHA were elevated with the increase in n-3 PUFA in the diets ($P < 0.006$).

Percent composition of each fatty acid, except palmitic acid (C16:0), in progeny chick spleen upon hatching reflected that of the egg yolk and significantly differed across treatments (Table 4). In comparison to the yolk fatty acids, a near two-fold increase of stearic acid (C18:0) was observed in the newly hatched chick spleens. The amount of PUFA was considerably increased in progeny spleens relative to PUFA levels in the egg yolk. Compared with yolk PUFA, the amounts of AA, EPA, and DHA in hatchling spleens were increased by 6 to 10, 5 to 6, and 0.5 to 2 folds, respectively. The increased incorporation of PUFA into the chick spleen was achieved at the expense of oleic acid (C18:1). This indicates a strong demand of long-chain n-6 and n-3 PUFA by the spleens of embryos or hatching chicks, and this demand was met by preferentially incorporating these fatty acids from egg yolk. Similar results have been reported in other tissues such as liver, heart, and brain (Cherian and Sim, 1992, 1993; Maldjian et al., 1995; Cerolini et al., 1996; Farkas 1996a,b).

After 2 wk on an identical diet (broiler starter), there were no differences among the treatment groups in levels of each saturated or monounsaturated fatty acid or in n-6 PUFA. Differences were detected among the treatments for the amount of DPA ($P < 0.03$), DHA ($P < 0.0003$), total n-3 PUFA ($P < 0.0003$), and n-6 to n-3 PUFA ratio ($P = 0.001$, Table 5). Broilers from hens given higher levels of n-3 PUFA in the diets retained more ($P < 0.05$) DPA and DHA in their spleens, with no differences observed between broilers from hens fed 2.5% SO plus 2.5% FO and those from hens fed 5% FO ($P > 0.05$). It is surprising that 4 wk after hatching, the levels of DHA in broiler spleens still differed ($P < 0.006$) at 1.05, 0.88, and 0.72% for broilers from hens fed 5% FO, 2.5% FO plus 2.5% SO, and 5% SO, respectively. No difference was detected in the other fatty acids. The FO group had higher ($P < 0.006$) levels of DHA than the SO group, suggesting a strong sequestering response of broiler spleen to DHA after hatching.

The present study showed that the level of DHA in the broiler immune system was affected by maternal supply and that this effect lasted for up to 4 wk after hatching. The levels of all other fatty acids did not differ among treatment groups at 4 wk. There is no literature available regarding the effect and duration of maternal n-3 fatty

TABLE 4. Fatty acid composition of the day-old broiler spleen

Fatty acids[2]	Diet[1] (% of total fatty acids) 5% SO	2.5% SO + 2.5% FO	5% FO	$P <$
C16:0	24.59 ± 0.59	26.33 ± 0.74	26.04 ± 0.50	NS
C18:0	16.01 ± 0.32[a]	15.88 ± 0.27[a]	14.20 ± 0.33[b]	0.021
C18:1	16.48 ± 1.16[b]	19.32 ± 1.01[b]	27.07 ± 0.91[a]	0.0001
C18:2n-6	14.55 ± 0.37[a]	13.63 ± 0.25[a]	7.40 ± 0.53[b]	0.0001
C20:3n-6	0.62 ± 0.04[b]	0.93 ± 0.05[a]	0.50 ± 0.05[b]	0.0001
C20:4n-6	18.30 ± 0.58[a]	10.90 ± 0.28[b]	5.07 ± 0.20[c]	0.0001
C22:4n-6	3.49 ± 0.18[a]	1.25 ± 0.04[b]	0.39 ± 0.04[c]	0.0001
C20:5n-3	0.00 ± 0.00[c]	2.65 ± 0.14[b]	6.28 ± 0.46[a]	0.0001
C22:5n-3	0.30 ± 0.03[c]	1.73 ± 0.13[b]	2.83 ± 0.21[a]	0.0001
C22:6n-3	1.59 ± 0.18[c]	5.90 ± 0.30[b]	8.33 ± 0.27[a]	0.0001
SAFA	41.28 ± 0.74	43.55 ± 0.74	41.89 ± 0.82	NS
MUFA	16.92 ± 1.11[b]	19.32 ± 1.01[b]	27.07 ± 0.91[a]	0.0001
PUFA	39.35 ± 0.20[a]	36.98 ± 0.62[b]	30.80 ± 0.36[c]	0.0001
n-6	37.46 ± 0.32[a]	26.71 ± 0.40[b]	13.36 ± 0.65[c]	0.0001
n-3	1.89 ± 0.20[c]	10.27 ± 0.32[b]	17.44 ± 0.42[a]	0.0001
n-6/n-3	20.44 ± 2.02[a]	2.61 ± 0.07[b]	0.77 ± 0.06[b]	0.0001

[a–c]For each fatty acid, values with different superscripts are significantly different ($P < 0.05$); values are means ± SEM (n = 5).

[1]SO = sunflower oil; FO = fish oil.

[2]SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-6/n-3 = the ratio of n-6 to n-3 PUFA.

acids alone on the DHA levels in the immune system and in other tissues of posthatching chicks. Some studies examined the effect of egg yolk PUFA on the DHA levels of the neural system and other tissues of the progeny upon hatching. It has been reported that DHA is preferentially mobilized from adipose tissue and uptaken by the developing neural tissues during incubation; the amount of DHA in the brain increases more than fivefold between Day 12 of development and hatching (Farkas et al., 1996a). The level of DHA in the hatchling brain is determined by its levels in the eggs from which the chicks hatched (Halle, 1999). These results are in agreement with the present study's findings on the effect of maternal supplies of n-6 and n-3 fatty acids on the n-3 PUFA profiles of newly hatched chicks. The duration of action and physiological relevance of maternal n-3 PUFA may be important to chicks in later life and should be elucidated in future research.

The wing web swelling responses to BSA in breeder hens were significantly suppressed by dietary n-3 fatty acids (Figure 1). Hens fed 5% FO had lower ($P = 0.023$) BSA-induced wing web swellings than those fed 2.5% FO plus 2.5% SO, and both groups had lower wing web swelling reactions to BSA ($P < 0.013$) than hens fed 5% SO. The level of n-3 PUFA in breeder hen diets also had a significant effect on the BSA-induced wing web swelling in the progeny up to 4 wk posthatch. At 2 wk of age, the broilers from hens fed 2.5% SO plus 2.5% FO had lower wing web swelling reactions to BSA than those from hens fed 5% FO ($P < 0.05$) and those from hens fed 5% SO ($P < 0.03$). No difference was observed between the two later groups, although broilers from hens fed 5% FO were 18% lower in wing web swellings than those from hens fed 5% SO. At 4 wk, BSA-induced wing web swelling responses in broilers from hens fed 2.5% FO diet did not differ from those hens fed a 5% FO diet. Both groups had lower ($P < 0.01$) BSA-induced wing web swellings than those from hens fed a 5% SO diet (Figure 2).

The broiler breeder hens were not exposed, either prior to or during the experiment, to diets containing meat meal. The progeny were fed meat meal free diets after hatching. The hens and chicks were not sensitized to BSA before the wing web swelling test. Therefore, the hens and chicks should not have BSA-specific T or B cells. The wing web swelling reaction to BSA might not be a delayed-type hypersensitivity reaction needing sensitization prior to the

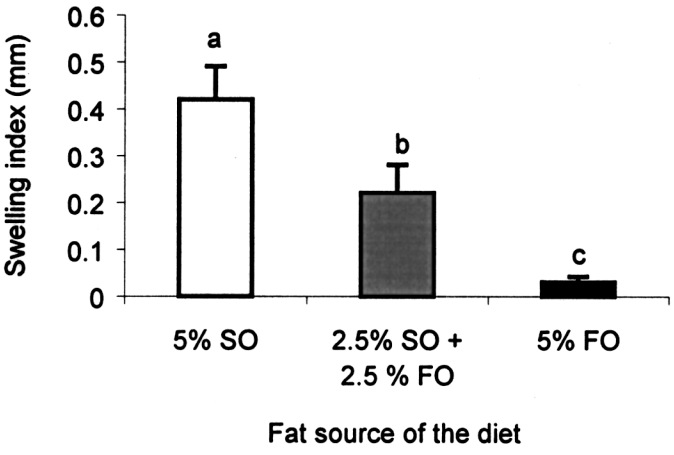

FIGURE 1. Effect of dietary n-3 fatty acids on BSA-induced wing web swellings in broiler breeder hens. The wing web swelling response was induced by subcutaneously injecting 0.2 mL of BSA solution (1 mg/mL in PBS) into two sites of the left wing web and 0.2 mL PBS into two sites of the right wing web. The BSA-induced wing web swelling response was presented by the swelling index, which was calculated as [swelling index = (thickness of left wing web 24 h following injection of BSA − initial thickness of left wing web) − (thickness of right wing web 24 h following injection of PBS − initial thickness of right wing web)]. Each bar represents mean ± SEM (n = 8). [a–c]Bars that do not have common letters are significantly different ($P < 0.03$). SO = sunflower oil; FO = fish oil.

TABLE 5. Fatty acids composition of 2-wk-old broiler spleen

Fatty acids[2]	Diet[1] (% of total fatty acids) 5% SO	2.5% SO + 2.5% FO	5% FO	$P <$
C16:0	24.35 ± 0.33	23.74 ± 0.28	23.42 ± 0.41	NS
C18:0	13.22 ± 0.57	13.97 ± 0.11	13.68 ± 0.19	NS
C16:1	2.31 ± 0.31	1.71 ± 0.07	1.65 ± 0.13	NS
C18:1	27.96 ± 1.45	26.86 ± 0.48	28.1 ± 0.53	NS
C18:2n-6	12.54 ± 0.18	12.62 ± 0.48	13.30 ± 0.21	NS
C20:3n-6	1.34 ± 0.11	1.44 ± 0.04	1.45 ± 0.09	NS
C20:4n-6	6.66 ± 0.52	6.51 ± 0.39	6.11 ± 0.38	NS
C22:4n-6	1.56 ± 0.15	1.32 ± 0.14	1.14 ± 0.08	NS
C18:3n-3	0.81 ± 0.18	0.64 ± 0.03	0.78 ± 0.08	NS
C20:5n-3	0.98 ± 0.10	1.26 ± 0.07	1.20 ± 0.05	NS
C22:5n-3	1.46 ± 0.12[b]	1.75 ± 0.04[a]	1.72 ± 0.03[a]	0.03
C22:6n-3	0.56 ± 0.10[b]	1.20 ± 0.05[a]	1.25 ± 0.10[a]	0.0003
SAFA	39.36 ± 0.85	39.68 ± 0.23	38.94 ± 0.52	NS
MUFA	32.71 ± 1.66	31.27 ± 0.58	32.24 ± 0.69	NS
PUFA	27.27 ± 0.84	28.16 ± 0.58	28.13 ± 0.62	NS
n-6	23.3 ± 0.76	23.14 ± 0.56	23.04 ± 0.63	NS
n-3	3.97 ± 0.18[b]	5.02 ± 0.05[a]	5.09 ± 0.16[a]	0.0003
n-6/n-3	5.87 ± 0.28[b]	4.61 ± 0.11[a]	4.54 ± 0.21[a]	0.0012

[a–b]For each fatty acid, values with different superscripts are significantly different ($P < 0.05$); values are means ± SEM (n = 5).

[1]SO = sunflower oil; FO = fish oil.

[2]SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-6/n-3 = the ratio of n-6 to n-3 PUFA.

swelling test (Parmentier et al., 1993). BSA is not considered inflammatory, and this response is probably not a local macrophage reaction. The BSA-induced wing web swelling is most likely due to the basophilic response and edema as characterized in the cutaneous hypersensitivity response to phytohemagglutinin (Kean and Lamont, 1994). The cutaneous basophil hypersensitivity response to phytohemagglutinin has long been used as a measurement of cell-mediated immunity in chickens (Kean and Lamont, 1994). Further experimentation is necessary to examine the exact nature of BSA-induced swelling responses.

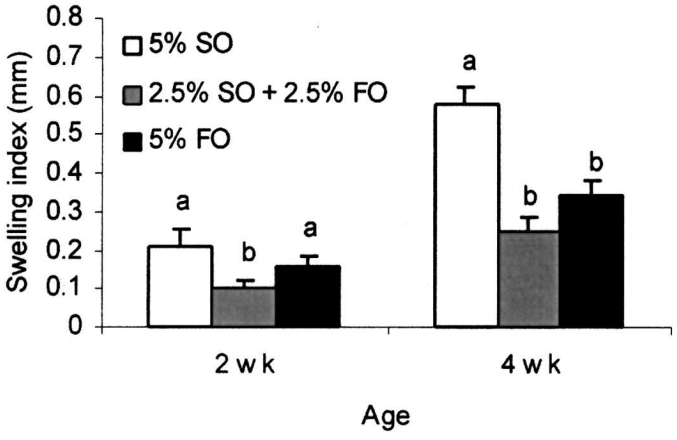

FIGURE 2. Effect of the maternal dietary n-3 fatty acids on BSA-induced wing web swellings in broilers at 2 and 4 wk of age. Broilers were fed identical starter (1 to 3 wk) and grower (4 to 6 wk) diets after hatching. The BSA-induced wing web swelling response was measured by subcutaneously injecting 0.2 mL of BSA solution (1 mg/mL in PBS) into two sites of the left wing web and 0.2 mL PBS into two sites of the right wing web. The BSA-induced wing web swelling response was presented by the swelling index, which was calculated as [swelling index = (thickness of the left wing web 24 h following the injection of BSA – the initial thickness of the left wing web) – (thickness of the right wing web 24 h following the injection of PBS – the initial thickness of the right wing web)]. Each bar represents mean ± SEM (n = 8). [a,b]Bars with different letters are significantly different ($P < 0.01$). SO = sunflower oil; FO = fish oil.

Suppressed cell-mediated immune responses by higher levels of n-3 PUFA have been reported in chickens (Fritsche et al., 1991; Wang 2000a,b). This effect might be, in part, due to reductions in levels of tissue AA and its eicosanoids. Prostaglandin E_2 and leukotriene B_4 derived from AA are potent lipid mediators regulating immune functions (Calder, 1997,1998). N-3 PUFA modifies both the quantity and diversity of eicosanoids, and eicosanoids produced from fish oil are less potent than those generated from AA (Billiar et al., 1988; Wallace et al., 2000). However, many cellular effects exerted by n-3 PUFA cannot be explained solely on the basis of alteration of substrate availability for eicosanoid biosynthesis (Hwang, 2000). Consuming n-3 PUFA resulted in a reduction in the production of cytokines such as interleukin-1, interleukin-2, and tumor necrosis factor-α, which are important in cell-mediated and inflammatory immune responses (Santoli and Zurier, 1989; Virella et al., 1989; Endres et al., 1993). Although not measured in this study, it has been reported that fish oil suppresses the release, relative to corn oil, of interleukin-1 and tumor necrosis factor-α in chicken (Korver and Klasing 1997; Korver et al., 1997).

Body weight gain and feed efficiency of the broilers were not affected, although BSA-induced wing web swelling responses were suppressed by adding fish oil (2.5 or 5%, wt/wt) to the breeder hen diets (data not shown). In the present study, the broilers were raised in a relatively clean environment and were not challenged with any pathogens. Previous studies (Allen et al., 1996; Danforth et al., 1997) have demonstrated that feeding diets containing 5 to 10% menhaden fish oil resulted in a decrease in both cecal

lesions and parasite numbers and an increase in weight gain in broiler chickens infected with *Eimeria tenella*. Another study has shown that low levels of fish oil (≤2% wt/wt) improved or did not change cell-mediated immune response, suppressed inflammation and improved feed efficiency and growth rate of chicks with moderate coccidial infection (Korver and Klasing, 1997). It is indicated that dietary inclusion of n-3 PUFA may be useful in controlling broiler coccidia. The present study indicates that maternal dietary n-3 PUFA in fish oil affects the n-3 fatty acids and BSA-induced wing web swelling responses in broilers of up to 4 wk of age. As broilers are raised commercially for 5 to 6 wk, immunoregulatory effects of maternal dietary n-3 fatty acids on progeny immune response could be adapted into health-promotion strategies of the broiler industry. However, for practical purposes, further studies are warranted to determine appropriate levels of maternal dietary n-3 PUFA and the n-6 to n-3 PUFA ratio for optimizing immune response, disease resistance and resilience.

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