Dietary Fish Oil Alters Specific and Inflammatory Immune Responses in Chicks[1,2,3]

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ABSTRACT Two experiments were designed to determine the effects of dietary (n-3) fatty acids and grain source on the growth-suppressive effects of the inflammatory response and indices of specific immunity. In Experiment 1, chicks were fed diets containing 0.5, 1, or 2 g/100 g of either corn oil or fish oil. In Experiment 2, chicks were fed diets containing up to 2 g/100 g of either fish oil, linseed oil or corn oil as the source of dietary fat, in either cereal grain- or corn-based diets. In each experiment, subsets of chicks within each dietary treatment were either vaccinated with infectious bronchitis virus (IBV) vaccine, injected with *Salmonella typhimurium* lipopolysaccharide (LPS), heat-killed *Staphylococcus aureus,* or remained noninjected. Increasing dietary fish oil, but not corn oil increased body weight and lessened the growth-suppressing effect of heat-killed *S. aureus* or *S. typhimurium* LPS. Increasing the concentration of dietary fish oil decreased febrile response, circulating hemopexin and metallothionein concentrations. Dietary fish oil resulted in decreased release relative to dietary corn oil of interleukin-1 by peritoneal macrophages. Although IBV titers were not significantly affected by dietary oil treatment, phytohemagglutination-induced wattle swelling was greater among chicks fed fish oil. In Experiment 2, the modulating effects of fish oil on the immune system were dependent on the type of grain used in the diet, with fish oil/cereal diets resulting in greater cell-mediated immunity and lower indices of inflammation than fish oil/corn diets. Inclusion of increasing amounts of fish oil in the diet improved performance, decreased indices of the inflammatory response and either improved or did not change indices of the specific immune response of growing chicks. J. Nutr. 127: 2039–2046, 1997.

KEY WORDS: • *chicks* • *fish oil* • *inflammatory response* • *(n-3) polyunsaturated fatty acids* • *immune system*

An inflammatory response can decrease feed consumption and muscle protein accretion, and increase metabolic rate, synthesis of acute phase proteins and organ mass relative to body mass (Klasing and Korver 1997, Roura et al. 1992). This change in the partitioning of nutrients away from growth and toward processes associated with the acute phase response is evident as a decrease in the efficiency of food use for growth. Strategies that minimize the diversion of nutrients away from growth and muscle deposition are important in animal production.

In mammals, the fatty acid composition of phospholipid membranes in immune cells can affect the degree of inflammatory response to a challenge with an immunogen, either in vitro (Billiar et al. 1988, Prescott 1984) or in vivo (German et al. 1987). Immune cells with membranes enriched in (n-3) polyunsaturated fatty acids (PUFA[5]) at the expense of (n-6) PUFA release lower amounts as well as less potent mediators of inflammation (Billiar et al. 1988, Prescott 1984). These mediators, the eicosanoids, are involved in the release and function of pro-inflammatory cytokines such as tumor necrosis factor α (TNF) (Scales et al. 1989), interleukin-1 (IL-1; Knudsen et al. 1986, Kunkel et al. 1987), and IL-6 (Navarra et al. 1992). Two eicosanoids important in the inflammatory response are prostaglandins of the E series (PGE) and leukotrienes of the B series (LTB).

Inclusion of fish oil in the diet has been shown to increase the proportion of (n-3) PUFA relative to (n-6) PUFA in the tissues of humans (Schmidt et al. 1991), rats (Billiar et al. 1988), mice (German et al. 1987, Whelan et al. 1991), and poultry (Chanmugam et al. 1992, Friedman and Sklan 1995, Fritsche et al. 1991b). Although most research of dietary oils has utilized high levels ($>$ 5 g/100 g) of inclusion in the diet, in mice the ratio of (n-3):(n-6) PUFA appears to be more important in modulating eicosanoid biosynthesis than the absolute concentration of (n-3) PUFA in the diet (Boudreau et al. 1991, Broughton et al. 1991). German et al. (1988)

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[5] Abbreviations used: IBV, infectious bronchitis virus; IL-1, interleukin 1; IL-6, interleukin 6; LPS, lipopolysaccharide; LTB, leukotriene B, PGE, prostaglandin E; PHA, phytohemagglutinin-P; PUFA, polyunsaturated fatty acid; TNF, tumor necrosis factor α.

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demonstrated that at high concentrations of dietary linoleic acid, fish oil supplementation had a minimal effect on leukotriene production relative to the same concentration of fish oil with lower concentrations of linoleic acid. In chickens fed corn-wheat-soy diets, increasing the (n-3):(n-6) PUFA ratio from 0.07 (the lowest possible in this type of diet) to 0.33 resulted in much greater liver (n-3) PUFA and much lower (n-6) PUFA (Korver 1997). Increases in the (n-3):(n-6) PUFA ratio to 0.66 and to 1.00 resulted in further, although more subtle, increases in hepatic (n-3):(n-6) PUFA ratio.

The enrichment of cell membrane (n-3) PUFA is associated with decreases in the inflammatory response, improvements in growth rate, and either increased or no change in specific immunity. The inclusion of fish oil in the diet of mammals appears to improve humoral immunity and ameliorate the suppression of the cellular immune response caused by PGE_2 (Fritsche et al. 1992, Schmidt et al. 1991).

The endogenous mediators of inflammation can themselves be involved in the pathogenesis of several diseases, including rheumatoid arthritis (Ridderstad et al. 1991), systemic lupus erythematosus (Das 1994) and atherosclerosis (Makheja 1992). Mice suffering from murine lupus nephritis and fed diets containing fish oil have decreased expression of renal IL-1, IL-6 and TNF mRNAs compared to those fed diets containing corn oil (Chandresekar and Fernandes 1994). The consumption of fish oil not only affects the release of regulatory mediators from various immune tissues, but also modulates the response of target tissues to those mediators. Rats fed diets containing fish oil have decreased pyrogenic responses to exogenous IL-1 (Cooper and Rothwell 1993) and anorexic responses to exogenous TNF (Mulrooney and Grimble 1993).

Fish oil concentrations greater than 2 or 3 g/100 g of diet may result in fish flavors in poultry meat and are impractical because of decreased consumer acceptance (Dean et al. 1969, Miller et al. 1967). However, lower dietary concentrations enrich poultry in (n-3) fatty acids for human consumption and might also be beneficial to the productivity of the chicken. The first experiment presented here was conducted to determine if the inclusion of low amounts of fish oil in the diets of fast-growing broiler chickens could lessen the negative impact of an experimental inflammatory response on their growth rate, feed consumption, and body weight gain per unit feed consumption. The experiment was designed to relate any changes in these parameters to indices of inflammatory and specific immune responses. The second experiment was designed to examine the relative efficacy of fish oil in diets with different carbohydrate sources on modulation of the inflammatory and immune responses.

MATERIALS AND METHODS

Birds and management. Male commercial Hubbard by Hubbard broiler chicks (A & M Hatchery, Santa Rosa, CA) were raised in Petersime brooder batteries with raised floors (Petersime Incubator Co., Gettysburg, OH) and provided a nutritionally complete corn and soybean meal–based starter diet with 13.4 kJ/g (Klasing and Barnes 1988) prior to the two experiments. When chicks were 3 d of age, experimental chicks were selected for uniform body weight from a twofold larger population and randomly assigned to dietary treatments. All experiments were approved by the University of California, Davis animal use committee.

Diets. The corn-soy experimental diets used in Experiment 1 were based on the NRC (1984) standard research reference diet for chicks to which either corn oil (Best Foods, CPC International, Englewood Cliffs, NJ) or menhaden oil (Zapata-Haynie, Reedville, VA) was added at 0.5, 1, or 2 g/100 g diet. **Table 1** shows composition and calculated (n-3):(n-6) PUFA ratio of each diet. Nine diets were used in Experiment 2 (**Table 2**), with menhaden, linseed (United States Biochemical Corp., Cleveland, OH), or corn oil as the source of dietary fat in either corn- or mixed cereal–based diets. Diets were kept isocaloric and isonitrogenous by adding appropriate amounts of corn starch and cellulose. Each of the six (Experiment 1) or nine (Experiment 2) experimental diets was fed to four pens of five chicks per pen.

Specific immune response. When the chicks were 14 d of age, they were vaccinated via intramuscular injection with 5 mg/kg body weight of infectious bronchitis virus vaccine (IBV; Bron-Newcavac-M, 10-006, Kirkegaard & Perry Laboratories, Gathersburg, MD). On d 28, venous blood was taken, and IBV antibody titers were determined by ELISA. Delayed-type hypersensitivity, a measure of cell-mediated immunity, was evaluated by the phytohemagglutinin-P– (PHA; Difco, Detroit, MI) induced wattle swelling assay as described by Klasing (1988). Briefly, chicks were injected on d 28 with 100 μL of PHA (500 μg/mL in phosphate-buffered saline) into the right wattle; only phosphate-buffered saline was injected into the left wattle. Twenty-four hours later, the thickness of each wattle was measured using a micrometer. The ratio of the thickness of the PHA-injected wattle to the thickness of the PBS-injected wattle is the wattle index. On d 29, Sephadex-elicited peritoneal macrophages were purified as described by Klasing and Peng (1987) and stimulated in vitro with heat-killed *S. aureus* to determine the capacity of these cells to produce interleukin-1 (IL-1). Briefly, macrophages were recruited by injecting into the peritoneal cavity of chicks 10 mL of a 50 g/L solution of Sephadex G-75 superfine (Pharmacia, Piscataway, NJ) in saline (9 g/L). Twenty-four hours later, cells were harvested from the peritoneum, washed, resuspended at 5×10^9 cells/L and cultured in RPMI 1640 medium containing per L 50 mL fetal bovine serum, 25 μmol 2-mercaptoethanol, 1×10^5 U penicillin, and 100 mg streptomycin. Cells were incubated at 42°C and 5% CO_2; after 6 h, nonadherent cells were washed off. Cells were stimulated to produce IL-1 by addition of 100 heat-killed *S. aureus*/macrophage for 4 h. Monolayers were washed twice with media and incubated an additional 16 h. *S. aureus* were grown in nutrient broth and washed three times before being killed at 85°C for 10 min. The bacteria were rewashed and suspended in medium. Culture supernatants were dialyzed (MW cut-off 2000) against phosphate-buffered saline, and biologically active IL-1 in the samples was measured by PHA-induced comitogenesis of thymocytes (Klasing and Peng 1987). Briefly, several thymic lobes were removed from chicks, placed in medium and teased apart using forceps. Debris was removed by sedimentation for 10 min, after which a single-cell suspension was collected and washed three times by centrifugation at $600 \times g$ for 10 min. Red blood cells were concentrated at the bottom of the centrifuge tube and discarded after each wash. Cells (50 μL of cell suspension) were incubated in 96-well plates at 2×10^6 cells per well. Fifty μL of cell culture supernatants and 50 μL of PHA (4 mg/L final concentration), and 50 μL treatments or medium were added resulting in a final volume of 200 μL. Cells were incubated at 42°C and 5% CO_2 for a total of 72 h; the final 18 h in the presence of 27 Bq [^{3}H]thymidine (20 μL, New England Nuclear, Boston, MA). Cells were harvested onto glass fiber filter mats using a Skatron cell harvester (Skatron Co., Sterling, VA). IL-1 activity is reported as the stimulation index, which is the ratio of [^{3}H]thymidine incorporated into DNA of thymocytes incubated with the IL-1 source plus PHA to [^{3}H]thymidine in thymocytes incubated in medium plus PHA.

Inflammatory response. A second group of chicks was fed the same six (Experiment 1) or nine (Experiment 2) diets in 12 pens of five chicks per diet, starting when chicks were 3 d of age. When chicks were 10 d of age, four pens per diet were injected intraperitoneally with 3 mL of a 100 mg/L solution of *S. typhimurium* LPS; four pens were injected with 3×10^9 heat-killed *Staphylococcus aureus*/kg body weight; four pens were not injected and served as controls. Injections were repeated when chicks were 12 and 14 d of age to stimulate an authentic infectious challenge. *S. typhimurium* LPS was reconstituted in saline (9 g/L) to 100 mg/L and sterilized by passing through a 0.45 μm filter. *S. aureus* were grown in nutrient broth, washed in saline, heat-killed at 85°C for 10 min and suspended in saline (9 g/L) at 10^{12} cells/L. When chicks were 15 d of age, they were bled, killed by cervical dislocation. Livers were removed, freeze-clamped in liquid nitrogen, and stored frozen until analysis.

Gain, feed intake, and feed conversion efficiency (gain ×

TABLE 1

Composition and calculated (n-3):(n:6) polyunsaturated fatty acid ratio of diets fed in Experiment 1[1,2]

	0.5% FO	1% FO	2% FO	0.5% CO	1% CO	2% CO
Ingredient						
Corn, *g/kg*	580	580	580	580	580	580
Soy meal (48.5% crude protein), *g/kg*	350	350	350	350	350	350
Corn starch, *g/kg*	50	40	20	50	40	20
Cellulose, *g/kg*	0	5	15	0	5	15
Fish oil, *g/kg*	5	10	20	0	0	0
Corn oil, *g/kg*	0	0	0	5	10	20
Calculated (n-3):(n-6) PUFA ratio	0.18	0.28	0.47	0.07	0.07	0.07
Calculated Composition, all diets						
ME, *kJ/g*				13.3		
Crude protein %				22.2		
Lys, %				1.2		
Met + Cys, %				0.94		

[1] With the exception of corn starch, cellulose, fish oil and corn oil, all ingredients, including vitamins and minerals were provided at NRC (1984) standard reference diet levels and chemical forms.

[2] Abbreviations used: CO, corn oil; FO, fish oil; ME, metabolizable energy; PUFA, polyunsaturated fatty acids.

feed^{-1}) of this second group of chickens were determined between days 10 and 15 of the experiment. Concentrations of the acute phase protein, hemopexin, in the plasma taken on the final day of the experiment were determined to give an index of the acute phase response. Hemopexin concentrations were determined by rocket gel electrophoresis using a rabbit anti-chicken hemopexin antibody. The concentration of the acute phase protein metallothionein in the liver was assessed by the ^{109}Cd affinity assay described by Eaton and Toal (1982). Cloacal temperature was determined 6 h following the first immunogen injection to provide an index of the responsiveness of the hypothalamus to cytokines released during the inflammatory stress.

Statistical analysis. For Experiment 1, data were analyzed by a three-way analysis of variance with oil source, oil concentration, and immunogen as main effects, and for their interactions using the general linear model procedure of the Statistical Analysis System (SAS) computer program (SAS Institute 1985). For Experiment 2, data were analyzed by a two-way analysis of variance with dietary treatment and immunogen as main effects, and for interactions using the general linear model procedure of SAS. When main effects due to dietary treatment, oil concentration, or immunogen were significant ($P < 0.05$), significant differences between main effect means were determined by the method of Tukey (Steel and Torrie 1980) using SAS. All dependent variables were also analyzed by a one-way ANOVA with 18 unrelated treatments (Experiment 1) or 27 unrelated treatments (Experiment 2). Single degree of freedom orthogonal contrasts (Steel and Torrie 1980) were made for indicated comparisons using the GLM procedure of SAS.

TABLE 2

Composition and calculated (n-3):(n-6) polyunsaturated fatty acid ratio of diets fed in Experiment 2[1,2]

	Cereal			*Corn*				*Corn*	
	1% FO	1.5% FO	2% FO	1.5% FO	2% FO	2% CO	Cereal 2% CO	2% LO	0.5% FO + 10% FM
Ingredient, *g/kg*									
Corn	80	80	80	515	515	515	80	515	560
Soy	350	350	350	385	385	385	350	385	225
Wheat[3]	330	330	330	0	0	0	330	0	0
Barley	150	150	150	0	0	0	150	0	0
Fish meal	0	0	0	0	0	0	0	0	100
Corn starch	40	30	20	10	0	0	20	0	10
Fish oil	10	15	20	20	20	0	0	0	5
Corn oil	0	0	0	0	0	20	20	0	0
Linseed oil	0	0	0	0	0	0	0	20	0
D,L-Methionine	0.25	0.25	0.25	0.22	0.22	0.22	0.25	0.22	0.05
L-Lysine · HCl	0.09	0.09	0.09	0.08	0.08	0.08	0.08	0.08	0
Cellulose					(to 1 kg)				
Calculated (n-3):(n-6) PUFA ratio	0.57	0.78	0.98	0.40	0.50	0.07	0.08	0.73	0.33
Calculated composition, all diets									
ME, *kJ/g*					11.7				
Crude protein, %					20.7				
Lys, %					1.23				
Met + Cys, %					0.95				

[1] Vitamins and minerals were provided at NRC (1984) standard reference diet levels and chemical forms; each diet contained: NaCl, 5 g/kg; choline chloride, 0.75 g/kg; $CaCO_3$, 10 g/kg; $CaHPO_4 \cdot H_2O$, 20 g/kg.

[2] Diets are described by supplemental oil type (FO, fish oil; CO, corn oil; LO, linseed oil; FM, fish meal) and grain type used in each diet.

[3] Yecoro Roho hard red winter wheat.

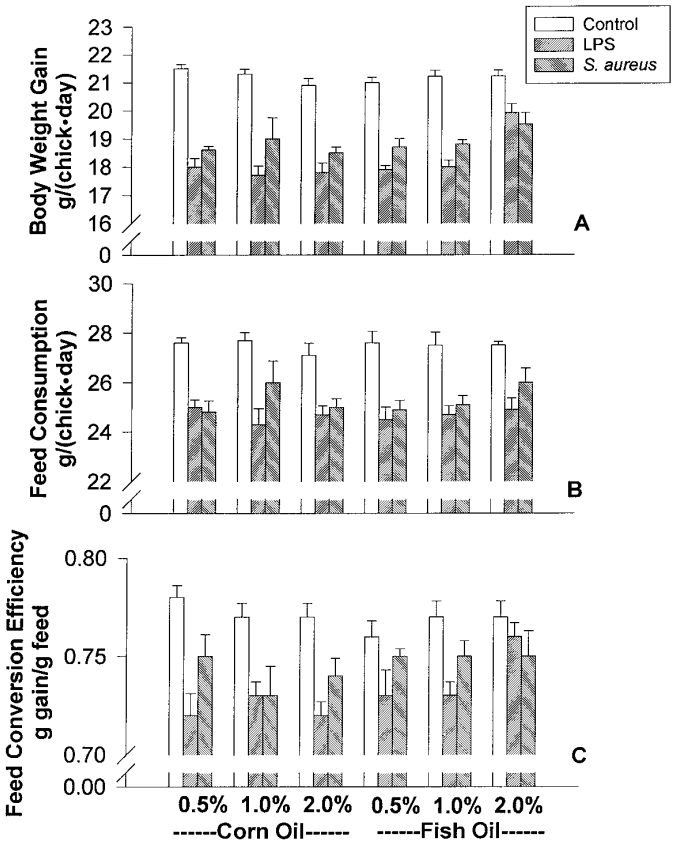

FIGURE 1 Effect of dietary oil source and immunogen challenge on growth rate, feed consumption and feed conversion efficiency of broiler chicks in Experiment 1. *Panel A:* Body weight gain (g/chick · day). Significant main effect and interaction *P* values: immunogen, $P < 0.01$; source by level, $P = 0.02$; source by level by immunogen, $P = 0.02$. *Panel B:* Feed consumption (g/chick · day). Significant main effect *P* value: immunogen, $P < 0.01$. *Panel C:* Feed conversion efficiency (g body weight gain/g feed consumed). Significant main effect *P* value: immunogen, $P < 0.01$. In each panel, individual bars represent the mean + SEM of four replicates.

RESULTS

Immunogen injection significantly ($P < 0.01$) decreased body weight gain, feed consumption, and feed conversion efficiency (**Fig. 1**). There was an interaction between oil concentration and source ($P = 0.02$) for body weight gain, where incremental increases in dietary oil concentration resulted in greater growth rate in the fish oil treatments but not in the corn oil treatments. Among chicks injected with LPS, those fed corn oil diets grew slower ($P = 0.01$ by orthogonal contrast) and tended to have lower feed efficiency ($P = 0.06$) than those fed fish oil diets. As the concentration of fish oil in the diet increased, the negative effect of immunogen treatment was lessened. However, increasing the level of corn oil in the diet did not have the same effect.

In Experiment 2, immunogen injection significantly decreased body weight gain ($P < 0.01$), feed consumption ($P = 0.04$), and feed conversion efficiency ($P < 0.01$; **Fig. 2**). There was a diet by immunogen interaction for gain ($P = 0.03$) and feed conversion efficiency ($P = 0.05$), and a nonsignificant trend for feed consumption ($P = 0.09$). For weight gain, this interaction is evident as an average decrease of 12.1% from control following LPS injection in chicks fed 2 g corn oil/100 g diet (cereal or corn diets) and only a 6.9% decrease for chicks fed either of the 2 g/100 g fish oil diets ($P < 0.01$ by orthogonal contrast). Among chicks fed the fish oil/cereal diets and injected with LPS, the 1, 1.5 and 2 g/100 g fish oil diets resulted in 11, 8, and 6% decreases in body weight gain relative to the respective uninjected controls. LPS injection of chicks fed corn diets containing either 1.5 or 2 g/100 g fish oil gained 9% less than the respective uninjected controls. LPS-injected chicks fed 2 g/100 g corn oil in corn- or cereal-based diets gained 13% and 9% less body weight than the respective noninjected controls. For feed conversion efficiency, the decrease due to LPS or *S. aureus* injection was significantly greater in chicks fed the corn oil diets than the fish oil diets ($P = 0.04$ by orthogonal contrast).

Injection of immunogen resulted in higher body temperature across all dietary treatments ($P < 0.01$; **Fig. 3**). There was a significant ($P = 0.05$) concentration by source interaction in which the febrile response to immunogen was lessened as fish oil in the diet increased, but remained fairly constant as corn oil in the diet increased. Hemopexin concentrations were 610% greater than control values ($P < 0.01$) following injection of immunogens in Experiment 1 (Fig. 3). The greater concentration of hemopexin due to LPS injection was augmented by increasing dietary corn oil from 0.5 to 2 g/100 g, but was reduced by increasing dietary fish oil ($P = 0.03$ by

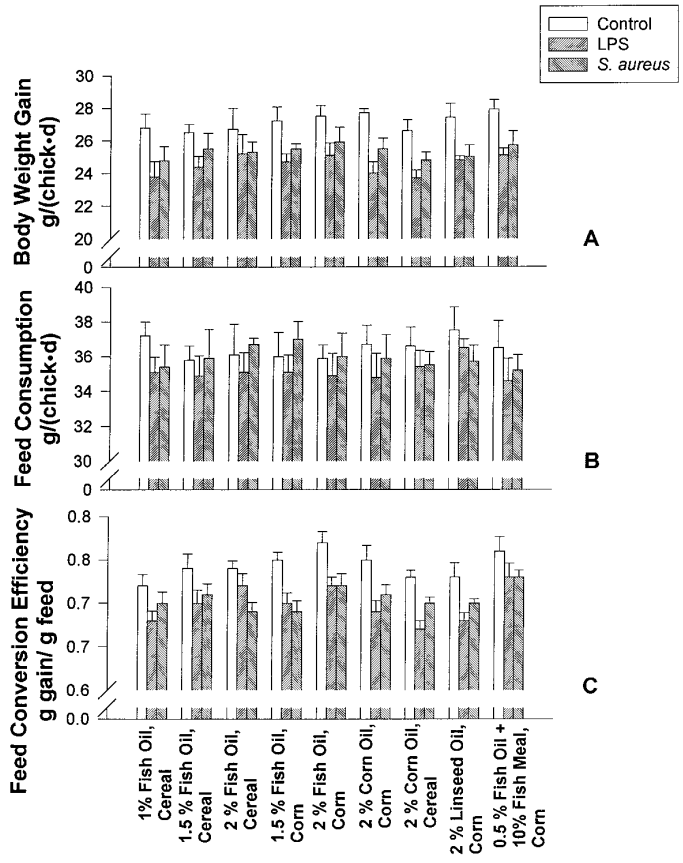

FIGURE 2 Effect of dietary oil source, grain type and immunogen challenge on growth rate, feed consumption and feed conversion efficiency of broiler chicks in Experiment 2. *Panel A:* Body weight gain (g/chick · day). Main effect and interaction *P* values: diet, $P = 0.09$; immunogen, $P < 0.01$; diet by immunogen, $P = 0.03$. *Panel B:* Feed consumption (g/chick · day). Main effect and interaction *P* values: diet, $P = 0.36$; immunogen, $P < 0.04$; diet by immunogen, $P = 0.09$. *Panel C:* Feed conversion efficiency (g body weight gain/g feed consumed). Main effect and interaction *P* values: diet, $P = 0.17$; immunogen, $P < 0.01$; diet by immunogen, $P = 0.05$. In each panel, individual bars represent the mean + SEM of four replicates.

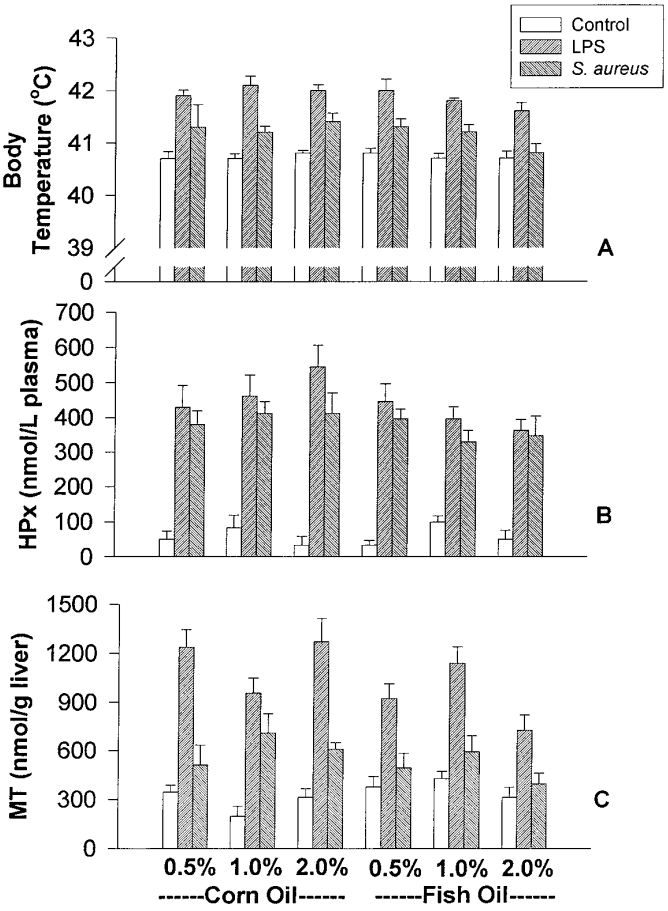

FIGURE 3 Effect of dietary oil source and immunogen challenge on indices of the inflammatory response of broiler chicks in Experiment 1. *Panel A:* Body temperature 6 h postinjection. Significant main effect and interaction *P* values: immunogen, $P < 0.01$; source by level, $P = 0.04$; source by level by immunogen, $P = 0.04$. *Panel B:* Plasma hemopexin (nmol/L plasma) 24 h after the third injection. Significant main effect and interaction *P* values: source, $P = 0.05$; immunogen, $P < 0.01$. *Panel C:* Hepatic metallothionein (nmol/g liver) 24 h after the third injection. Significant main effect and interaction *P* values: source, $P = 0.04$; immunogen, $P < 0.01$; source by level, $P = 0.01$; source by immunogen, $P = 0.01$; source by level by immunogen, $P = 0.01$. In each panel, individual bars represent the mean + SEM of four replicates. Abbreviations: Hpx = hemopexin; MT = metallothionein.

orthogonal contrast). Metallothionein concentrations were 125% greater ($P < 0.01$) in response to immunogen injection, and the effect was dependent on the dietary oil type ($P = 0.01$). Metallothionein concentrations were not different due to oil source in noninjected birds ($P = 0.21$ by orthogonal contrast), but were significantly less in chicks injected with LPS when they were fed fish oil than when they were fed corn oil ($P < 0.01$). There was a significant oil concentration by oil source interaction for metallothionein concentrations ($P < 0.01$). Concentrations of hepatic metallothionein in chicks fed corn oil increased as dietary corn oil increased, while increasing fish oil from 0.5 to 2 g/100 g diet resulted in lower average metallothionein concentrations.

Body temperature was lower in chicks fed the cereal-based diets than in those fed the corn-based diets (41.03°C vs. 41.26°C, $P < 0.01$ by orthogonal contrast), and body temperature was significantly higher due to immunogen injection ($P < 0.01$; **Fig. 4**). For chicks fed 2 g/100 g oil diets, the febrile response to LPS or *S. aureus* was greater with corn oil than with fish oil ($P < 0.01$ by orthogonal contrast). Hemopexin concentrations were elevated 383% ($P < 0.04$) by immunogen injection, and this effect was greater in chicks fed diets supplemented with corn oil than those fed diets supplemented with fish oil ($P < 0.01$ by orthogonal contrast). Metallothionein concentrations were 619% greater ($P < 0.01$) in response to immunogen injection; this effect was greater in chicks fed corn oil diets than those fed fish oil diets ($P < 0.01$ by orthogonal contrast).

Antibody titers to infectious bronchitis virus were not altered by dietary treatment in Experiment 1 (**Table 3**). PHA-induced wattle swelling was greater ($P < 0.01$) when chicks were fed fish oil compared to corn oil diets. The release of IL-1 by peritoneal macrophages was lower ($P < 0.01$) in chicks fed fish oil compared to those fed corn oil. There was a significant oil concentration by oil source interaction ($P = 0.02$) for IL-1. Increasing corn oil from 0.5 to 2 g/100 g diet resulted in greater IL-1 release, while increasing fish oil from 0.5 to 2 g/100 g diet resulted in lower IL-1 release.

Antibody titers to infectious bronchitis virus were not altered by dietary treatment in Experiment 2 (**Fig. 5**). PHA-induced wattle swelling was affected by dietary treatment ($P < 0.01$). The 2 g/100 g linseed oil/corn diet resulted in the

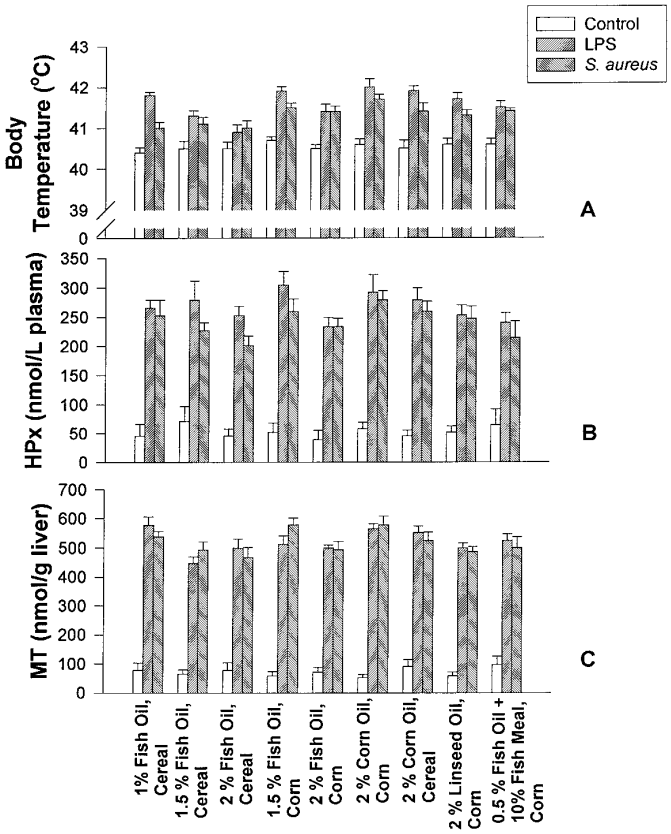

FIGURE 4 Effect of dietary oil source, grain type and immunogen challenge on indices of the inflammatory response of broiler chicks in Experiment 2. *Panel A:* Body temperature 6 h postinjection. Main effect and interaction *P* values: diet, $P = 0.11$; immunogen, $P < 0.01$; diet by immunogen, $P = 0.11$. *Panel B:* Plasma hemopexin (nmol/L plasma) 24 h after the third injection. Main effect and interaction *P* values: diet, $P = 0.27$; immunogen, $P < 0.01$; diet by immunogen, $P = 0.04$. *Panel C:* Hepatic metallothionein (nmol/g liver) 24 h after the third injection. Main effect and interaction *P* values: diet, $P = 0.16$; immunogen, $P < 0.01$; diet by immunogen, $P = 0.06$. In each panel, individual bars represent the mean + SEM of four replicates. Abbreviations: Hpx = hemopexin; MT = metallothionein.

TABLE 3

Effect of dietary oil source on indices of specific immunity and inflammatory responses of broiler chicks (Experiment 1)

Oil source	Amount	Anti-IBV[1] titer	Wattle index[2]	IL-1[3] stimulation index
Corn oil	0.5%	0.72	2.0	2.4
	1.0%	0.75	2.0	2.3
	2.0%	0.68	2.1	2.8
Fish oil	0.5%	0.75	2.2	2.1
	1.0%	0.72	2.2	2.1
	2.0%	0.77	2.4	1.8
SEM		0.03	0.11	0.18
P values				
Source		0.28	<0.01	<0.01
Level		0.88	0.19	0.84
Source by level		0.22	0.75	0.02

[1] Infectious bronchitis virus antibody (IBV) titer ($n = 4$). Chicks were vaccinated at 14 d of age with IBV, and antibody titers at d 28 were determined by ELISA (arbitrary units).

[2] Wattle index is the ratio of phytohemagglutinin (PHA)-induced swelling at 24 h postinjection relative to the vehicle-injected contralateral wattle ($n = 4$).

[3] Biological activity if interleukin-1 (IL-1) expressed as stimulation index (SI; $n = 4$) Cells were cultured for 18 h in the presence of *S. typhimurium* lipopolysaccharide, and the supernatants were assayed via thymocyte comitogenesis for IL-1 activity. SI is the ratio of [^{3}H]-thymidine incorporated into the DNA of thymocytes in the presence of the IL-1 source and PHA to the [^{3}H]thymidine incorporated into the DNA of thymocytes in the presence of PHA.

greatest, and the 2 g/100 g fish oil/corn diet the lowest wattle index. The fish oil/cereal diets tended ($P = 0.07$) to result in greater wattle indices than the fish oil/corn diets. Release of IL-1 by stimulated peritoneal macrophages was also affected by dietary treatment ($P = 0.03$). Chicks fed the 2 g/100 g fish oil/corn diet had the greatest, and those fed the 1.5 g/100 g fish oil/cereal diet had the lowest release of IL-1 activity.

DISCUSSION

We found that injection of growing chicks with inflammatory immunogens decreased their rate of body weight gain, feed intake, and feed conversion efficiency. These effects have been observed previously in several other studies using chickens (Benson et al. 1993, Klasing et al. 1987, Roura et al. 1992, Takahashi et al. 1995), pigs (McCracken et al. 1995), and rats (Peisen et al. 1995). When chicks were challenged with either LPS or *S. aureus,* the inclusion of fish oil in the diet partially mitigated the decrease in body weight gain and feed conversion efficiency. In Experiment 1, a bacterial challenge simulated by injecting LPS resulted in about a 15% decrease in the rate of gain of chicks consuming 2 g corn oil/100 g diet. This effect was lessened by feeding 2 g fish oil/100 g diet, resulting in only 10% lower rate of weight gain. In the second experiment, the efficacy of fish oil was examined with two different dietary backgrounds, either cereal or corn. Menhaden oil was effective at ameliorating LPS-induced growth depression, and this interaction was more pronounced with the cereal diets than the corn diets. Possibly this was due to the lower level of (n-6) fatty acids, including linoleic acid, found in the cereal diets relative to the corn diets, however the slightly slower growth rates in the absence of a challenge in cereal fed chicks could also contribute to this observation.

Our purpose in injecting nonreplicating immunogens on three alternate days was to simulate an infectious challenge. This apparently was accomplished because injection of either immunogen caused significantly higher body temperature and concentrations of the acute phase proteins hemopexin and metallothionein in chicks fed all dietary treatments. As with rate of gain, the magnitude of these acute phase responses was dependent upon either the concentration of oil in the diet or the source of oil. In general, the responses were greatest at the highest levels of corn oil and least at the highest level of fish oil.

Interleukin-1 induces fever (Dinarello 1988), and along with IL-6 and TNF, the synthesis of acute phase proteins such as hemopexin (Baumann and Gauldie 1994) and metallothionein (Bremner and Beattie 1990, Klasing 1984). In Experiment 1, the in vitro release of IL-1 from macrophages isolated from chicks fed fish oil was less than that from corn oil–fed

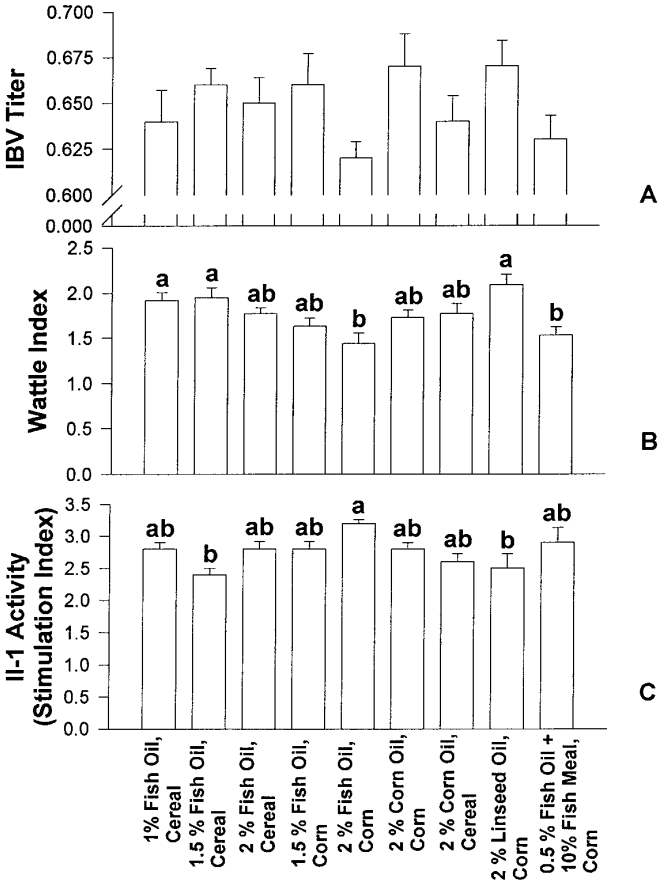

FIGURE 5 Effect of dietary oil source, grain type and immunogen challenge on indices of the immune response of broiler chicks in Experiment 2. *Panel A:* Circulating levels of antibodies to infectious bronchitis virus (IBV). Chicks were vaccinated at 14 d of age with IBV, and antibody titers at d 28 were determined by ELISA. Main effect *P* value: diet, $P = 0.19$. *Panel B:* Wattle index, where index is the ratio of phytohemagglutinin (PHA)-induced swelling at 24 h postinjection of the injected wattle relative to the vehicle-injected contralateral wattle. Main effect *P* value: diet, $P < 0.01$. *Panel C:* In vitro release of interleukin-1–like (IL-1) activity from sephadex-elicited peritoneal macrophages, expressed as stimulation index (SI). Cells were stimulated with *S. aureus* for 18 h, and the supernatants were assayed via thymocyte comitogenesis bioassay for IL-1 activity. SI is the ratio of [^{3}H]thymidine incorporated into the DNA of thymocytes in the presence of the IL-1 source and PHA to the [^{3}H]thymidine incorporated into the DNA of thymocytes in the presence of PHA. Main effect *P* value: diet, $P = 0.03$. In each panel, individual bars represent the mean + SEM of four replicates. Different letters indicate means differ significantly, $P < 0.05$.

chicks. Thus it is likely that the blunted acute phase response observed in vivo in fish oil–fed chicks was at least partly due to lower levels of inflammatory cytokines such as IL-1. Dietary (n-3) fatty acids have been shown to decrease interleukin-1 and tumor necrosis factor production by cultured human mononuclear cells (Endres et al. 1989). The mechanism by which (n-3) fatty acids specifically decrease the inflammatory response was not investigated in these experiments. Increasing the (n-3):(n-6) concentration increases the (n-3) content of membrane phospholipids of both target and effector cells. This in turn may result in decreased pro-inflammatory signals released by effector cells, and may also decrease responsiveness of target cells to pro-inflammatory signals.

The inflammatory response is thought to be the major component of the immune response that disrupts growth-related physiology, resulting in slower and less efficient growth during many clinically important diseases. In mammals and chicks, inflammatory cytokines decrease appetite and skeletal muscle protein accretion, and stimulate T cell proliferation and metabolic rate (Dinarello 1988, Klasing 1994). In our control birds, which were raised in environments that were extremely clean with minimal exposure to infectious challenges, there was no benefit or detriment in terms of growth to enriching the diet with (n-3) fatty acids. When the birds were challenged, however, the fish oil diets resulted in greater growth rates than did the corn oil diets. Thus, in practical poultry husbandry, fish oil may benefit growth when the birds are challenged by pathogens. Additionally, when birds are reared in commercial-type environments with the build-up of dust, dander, and feces, the inflammatory response is constantly stimulated. These nonpathogen environmental immunogens increase the level of the catabolic cytokine, IL-1, altering the birds' metabolism and redirecting nutrients away from growth and toward an inflammatory response (Roura et al. 1992). Under practical poultry production conditions, fish oil might be fed to minimize the catabolic effect of both pathogens and environmental immunogens by decreasing production of pro-inflammatory cytokines and acute phase proteins, permitting a higher growth rate.

Increasing dietary (n-3) fatty acids in Experiment 1 resulted in greater cell-mediated immunity as determined by the wattle delayed-type hypersensitivity assay. In Experiment 2, dietary fish oil tended to result in greater cell-mediated immunity when included in cereal-based diets than in corn-based diets. The fish oil/corn diets had lower (n-3):(n-6) PUFA ratios and resulted in greater cell-mediated immunity than the fish oil/cereal diets. The corn oil/corn diets had the lowest (n-3):(n-6) ratio, and yet chicks fed these diets had wattle indices that were intermediate to the above two types of fish oil diets. Thus, the effect of fish oil on wattle index appears to be dependent on the type of grain used in the diet, and the effect of diet type on cell-mediated immunity is not solely due to the dietary (n-3):(n-6) PUFA ratio.

In our study, moderate levels (1–2 g/100 g) of fish oil in the diet were utilized, but Fritsche et al. (1991a) reported that chicks fed a diet containing 7 g menhaden oil/100 g diet have higher antibody responses to sheep red blood cells than did chicks fed the same concentration of either lard, corn oil or canola oil. Cellular immune response as measured by antibody-dependent cell cytotoxicity of splenocytes is decreased in broilers fed 7 g fish oil versus those fed 7 g corn oil/100 g diet, although cytotoxicity of peripheral blood leukocytes is not affected by dietary treatment (Fritsche and Cassity 1992). Thus, high levels of dietary fish oil apparently have different immunomodulatory effects than lower levels. In mammals, diets containing fish oil either improve, decrease, or do not affect indices of specific immunity depending on the index of immune function, the amount of fish oil inclusion in the diet, and the concentration of dietary fat. Anti-sheep red blood cell antibody responses of rats fed 17 g fish oil + 3 g corn oil/100 g diet and either 30 or 90 mg vitamin E/100 g diet are significantly higher than corn oil–fed rats fed the same amounts of vitamin E (Fritsche et al. 1992). In noninfected mice, feeding a high (n-3) PUFA diet (20 g fish oil/100 g diet) results in the greatest percentage and number of T cells, but in *Listeria*-infected mice, this diet results in the lowest percentage of T cells in the peritoneum when compared to mice fed the same concentration of sunflower oil and coconut oils. B cell populations are not affected by dietary fat in noninfected mice, but the fish oil diet results in the highest percentage of B cells in infected mice (Huang et al. 1992). Splenocyte natural killer cell activity of mice fed 10 g fish oil/100 g diet is decreased 25% compared to that of mice fed a the same concentration of corn oil, although cell-mediated cytotoxicity of cytotoxic T lymphocytes and peritoneal cells is not affected (Fritsche and Johnston 1990). Level of inclusion appears to play a role in the effect of fish oil, since this oil is immunosuppressive in the host vs. graft model in mice only at high concentrations (10 g/100 g of diet) (Hinds and Sanders 1993). In humans, inclusion of fish oil at 0.54% of total energy in a low fat diet decreases T cell proliferation in response to Concanavalin A and PHA, while inclusion of only 0.13% of calories as fish oil in a similar diet results in an increase in the same indices. Delayed-type hypersensitivity is decreased versus baseline at the higher level of fish oil, but there was no change at the low level of fish oil (Meydani et al. 1993).

The results of these experiments give insight into a potential dietary method to decrease losses in growth rate, feed consumption, and feed conversion efficiency that might occur during infectious challenges. The modulation in sensitivity to an infectious challenge as measured by weight gain in our experiments appears to be due to a shift in the immune response away from the inflammatory response and toward humoral and/or cell-mediated responses. Indeed, indices of the inflammatory response were lower in fish oil–fed birds, while indices of specific immunity were either unchanged or greater among chicks fed fish oil in Experiment 1. The results of Experiment 2 demonstrate that the composition of the diet to which fish oil is added can also play a role in modulating the responsiveness to immune challenges. The implications of such a shift in the resistance of chickens to commercially relevant infectious challenges needs to be investigated. The inflammatory response is the first line of defense against novel pathogens, but cells and mediators of the inflammatory response have been implicated in the pathology of many poultry diseases, including coccidiosis (Trout and Lillehoj 1993) and *S. enteritidis* (Kogut et al. 1995, Tellez et al. 1994). The net benefit of immunomodulatory nutrients such as fish oil in natural disease challenges remains to be characterized. Under the conditions of these experiments, lessening the inflammatory response improved performance characteristics of broiler chickens.

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