

Lipid Measurements in Chickens Fed Different Combinations of Chicken Fat and Menhaden Oil

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Presently, the specific level of dietary fish oil necessary to decrease plasma triacylglycerols in humans or poultry is not known. The experiment described herein was conducted to evaluate the effects of varying levels of menhaden oil on plasma triacylglycerols in broiler chickens. Six lipid treatments consisting of chicken fat/menhaden oil combinations were fed at 50 g/kg of diet. There were no significant differences in body, liver, heart, and abdominal fat pad weights or in total liver lipids and liver triacylglycerols due to the dietary treatments. As the levels of dietary $n - 3$ fatty acids and the polyunsaturate:saturate ratios increased, the amounts of triacylglycerols decreased in the plasma and in the very low density plus low density lipoprotein (VLDL + LDL) fractions. On the other hand, plasma triacylglycerol levels increased as the dietary $n - 6$ fatty acids increased. The dietary intake of $n - 3$ polyunsaturates in menhaden oil led to elevated levels of these fatty acids in the chicken tissues evaluated.

INTRODUCTION

Coronary heart disease is a major health problem in developed countries. Dietary lipids are considered to be one of the risk factors that can predispose individuals to atherosclerosis and coronary heart disease (Jones, 1974; WHO, 1982; Surgeon General's Report on Nutrition and Health, 1988).

Dietary supplementation with polyunsaturated $n - 3$ fatty acids, such as those found in fish oil, were shown to decrease plasma triacylglycerols (Bang et al., 1971; Boberg et al., 1986) and reduce postprandial triglyceridemia (Harris et al., 1988) in humans. In a recent review, Harris (1989) summarized the effects of fish oil on (1) plasma lipid and lipoprotein levels, (2) plasma lipid and lipoprotein composition, and (3) lipoprotein metabolism. In general, consumption of fish oils, compared with other food lipids, depressed plasma triacylglycerol and total cholesterol levels while LDL and HDL cholesterol concentrations rose slightly.

Animal studies were conducted to further examine the effects of dietary $n - 3$ fatty acids on lipid metabolism. It appears that the absorption and transport of $n - 3$ fatty acids are similar to that of other long-chain polyunsaturated fatty acids in animals and humans (Nelson and Ackman, 1989). When rats were fed salmon oil, they had decreased plasma triacylglycerols compared with those fed lard (Nalbone et al., 1989). Wong et al. (1984) found that rats fed fish oil had reduced plasma triacylglycerols, liver lipogenesis, and very low density lipoprotein (VLDL) triacylglycerol secretion rates compared with those fed safflower oil. Mature roosters fed fish oil had lowered VLDL triacylglycerol secretion rates and plasma triacylglycerol levels compared with roosters fed corn oil (Daggy et al., 1987).

Simopoulos (1988) has proposed that the high-fat diets of humans in Western-industrialized countries are deficient in $n - 3$ fatty acids. The $n - 6:n - 3$ ratio in this type of diet ranged from 10:1 to 14:1 compared with 1:1 for diets consumed by wild animals. She recommended striving for a ratio of 1:1 in Western-industrialized diets to correct the $n - 6:n - 3$ fatty acid imbalance and thereby reduce the

arachidonic acid metabolites that may lead to coronary thrombosis.

The degree of saturation in dietary fats also plays a role in the development of atherosclerosis (Keys et al., 1957; FAO/WHO, 1977). The Food and Agriculture Organization (1977) promotes increasing the ratio of polyunsaturated to saturated fatty acids (P:S) in human diets to prevent atherosclerosis and coronary heart disease. Consumption of highly unsaturated $n - 3$ fatty acids in fish oils would help to reduce the concentration of saturated fatty acids in plasma.

The specific level of dietary fish oil necessary to decrease plasma triacylglycerols in humans or poultry is not known. The present study was conducted to determine the level of $n - 3$ fatty acids required to decrease plasma triacylglycerols in chickens. The chicken was selected as the animal model for this experiment because liver is the primary site for fatty acid synthesis, as it is in humans (O'Hea and Leveille, 1969; Guyton, 1986). Dietary lipid sources, chicken fat (CF) and menhaden oil (MO), were fed in combinations that provided a wide range of $n - 6$ and $n - 3$ fatty acids and P:S fatty acid ratios. Chicken fat is relatively high in saturates and $n - 6$ fatty acids (Pereira et al., 1976), while MO is high in $n - 3$ and long-chain polyunsaturated fatty acids (Phetteplace and Watkins, 1989; Ackman, 1982). Chicken fat provided a $n - 6:n - 3$ ratio of 24:1, while menhaden oil provided a $n - 6:n - 3$ ratio of 0.1:1. The P:S ratios for CF and MO were 0.72 and 1.38, respectively.

MATERIALS AND METHODS

Animals and Diets. Seventy-two male broiler chicks were individually wing-banded, weighed, and randomly placed in a temperature-controlled battery brooder with raised wire floors. A practical basal diet (Table I) was fed containing one of six chicken fat/menhaden oil combinations (CF:MO w/w) at 50 g/kg of diet. The CF:MO treatments were (1) 100:0, (2) 80:20, (3) 60:40, (4) 40:60, (5) 20:80, and (6) 0:100. The CF was a commercial feedfat source prepared by Holly Farms Industries Inc. (Wilkesboro, NC), and the MO, which was rich in 20:5 n 3 and 22:6 n 3, was supplied by Zapata Haynie Corp. (Reedville, VA). The basal diet was formulated to meet or exceed the nutrient requirements for the growing chick (National Research Council, 1984). Feed and water were offered ad libitum, and the chickens were maintained on continuous light (24 h light:0 h dark).

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Table I. Composition of the Basal Diet

item	amount, g/kg	item	amount, g/kg
ground wheat	394	CaCO ₃	10
soybean meal	300	vitamin premix ^b	10
ground corn	199	mineral premix ^c	8
lipid source ^a	50	DL-methionine	4
polypbos	25		
(Ca, 31%; P, 18%)			
Calculated Nutrient Analysis			
metabolizable energy		3069 kcal/kg	
crude protein		200 g/kg	

^a One of six lipid combinations of chicken fat/menhaden oil: (1) 100:0; (2) 80:20; (3) 60:40; (4) 40:60; (5) 20:80; (6) 0:100. ^b Contained the following (mg/kg of diet) unless otherwise noted: vitamin A palmitate, 2000 IU; vitamin D₃, 400 ICU; DL- α -tocopherol acetate, 20 IU; menadione-sodium bisulfite, 1.0; riboflavin, 3.6; pantothenic acid, 10.0; niacin, 27.0; vitamin B₁₂, 0.009; choline chloride, 900; folic acid, 0.55; thiamin, 1.8; pyridoxine, 3.0; biotin, 0.4; ethoxyquin (66%), 200. ^c Contained the following (mg/kg of diet): iodized NaCl, 4000; MnSO₄·H₂O, 184.6; ZnO, 50; FeSO₄·7H₂O, 400; CuSO₄·5H₂O, 31.5; KIO₃, 0.3; Na₂SeO₃, 0.32.

The diets containing MO were prepared frequently by adding the oil to the basal ingredients, and the finished diets were stored between 0 and 6 °C until fed. Fresh feed was added to the feeders every 48 h.

Sample Collection. Blood samples for triacylglycerol analysis were obtained at 42 and 45 days of age via the brachial vein. Blood was collected into an EDTA-coated (2 mg of EDTA/mL of blood) syringe and kept on ice until centrifugation. On day 45, chickens were killed by cervical dislocation following the blood sampling. The abdominal fat pad, liver, and heart were surgically removed, weighed, and kept on ice until frozen at -20 °C for later lipid analyses. A total of six chickens (fed-state) per dietary treatment were utilized for all analyses.

Analytical Procedures. Blood was centrifuged at 1000g for 20 min. The plasma was removed and stored at 4 °C until analyzed. Triacylglycerol content of plasma (Biggs et al., 1975) and very low density plus low density lipoprotein (VLDL + LDL) fractions (Whitehead and Griffin, 1982) were determined in duplicate. Liver triacylglycerols were determined by first extracting the tissue with chloroform/methanol (2:1, v/v) according to the method of Folch et al. (1957), evaporating the extracted lipid to dryness, adding 2 mL of heptane, and then following the procedure of Biggs et al. (1975). Triolein was purchased from Nu Chek Prep (Elysian, MN) and prepared as the standard. Triacylglycerol concentrations (micromoles per milliliter) were determined from a standard curve. Plasma triacylglycerol levels were expressed as micromoles per milliliter of plasma and micromoles per gram of liver.

Total liver lipids were determined gravimetrically by extraction with chloroform/methanol (2:1 v/v) as described by Folch et al. (1957). Lipids for fatty acid analyses were obtained from four chickens per treatment and extracted following the procedure of Folch et al. (1957). Prior to fatty acid analysis by capillary gas-liquid chromatography (GLC), the lipid extract was saponified and methylated by using 12% boron trifluoride as described by Metcalfe et al. (1966).

Individual fatty acid concentrations in lipid sources and tissue lipids were measured by using a gas chromatograph (Hewlett-Packard Model 5890A) equipped with a DB 225 fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.15 μ m) purchased from J & W Scientific Co. (Rancho Cordova, CA). The initial oven temperature (196 °C) was maintained for 12 min and then increased at a rate of 0.9 °C/min to a final temperature of 214 °C. Total run time was 40 min. The injection port and flame ionization detector temperatures were 250 °C. Nitrogen was used as the makeup gas for the detector and helium served as the carrier gas.

An external standard was prepared from triacylglycerols and methylated fatty acids purchased from Nu Chek Prep. The external standard was used to determine retention times for each fatty acid and to develop the calibration table. Prior to meth-

Table II. Dietary Lipid Fatty Acid Compositions (Grams/100 g of Fatty Acid Methyl Esters) for Varying Combinations of Chicken Fat/Menhaden Oil^a

fatty acid	chicken fat/menhaden oil					
	100:0	80:20	60:40	40:60	20:80	0:100
16:0	23.05	22.01	20.93	19.95	18.70	17.62
16:1n7	8.41	8.96	9.53	9.98	10.52	11.00
17:0	0.11	0.55	0.97	1.37	1.84	2.25
18:0	5.41	4.96	4.50	4.09	3.52	3.07
18:1	41.07	35.24	29.28	23.36	17.12	10.95
t18:2n6	ND ^b	0.09	0.10	0.10	0.10	0.10
18:2n6	18.92	15.46	11.97	8.43	4.83	1.19
18:3n6	0.28	0.34	0.42	0.31	0.29	0.38
18:3n3	0.82	0.84	0.88	0.90	0.92	0.96
20:0	ND	0.03	0.12	0.23	0.26	0.32
20:1n9	0.31	0.50	0.69	0.87	1.05	1.24
20:2n6	ND	0.10	0.10	0.14	0.14	0.15
20:3n6	0.19	0.21	0.22	0.23	0.25	0.27
20:4n6	0.39	0.50	0.63	0.73	0.85	0.97
20:5n3	ND	3.10	6.23	9.19	12.44	15.52
22:0	ND	ND	ND	ND	0.06	0.14
22:1n9	ND	ND	ND	ND	0.07	0.17
22:4n6	ND	0.03	0.03	0.17	0.16	0.14
22:5n6	ND	ND	0.12	0.18	0.31	0.34
22:5n3	ND	0.59	1.11	1.63	2.16	2.69
22:6n3	ND	1.97	3.91	5.73	7.75	9.68
others ^c	0.92	2.58	4.16	5.69	7.29	8.90
total saturates	28.56	27.56	26.52	25.64	24.38	23.40
total unsaturates	20.60	23.24	25.72	27.75	30.21	32.39
total n - 6	19.79	16.74	13.58	10.29	6.94	3.54
total n - 3	0.82	6.50	12.14	17.46	23.27	28.85
n - 6:n - 3	24.22	2.58	1.12	0.59	0.30	0.12
P:S	0.72	0.84	0.97	1.08	1.24	1.38

^a Values represent analysis of four lipid samples per treatment. ^b ND, not detected. ^c The following fatty acids were identified in the lipid samples: 12:0, 14:0, 14:1, and 15:0.

ylation of the external standard and the tissue lipid extracts, a known amount of internal standard (methylated heptadecenoic acid) was added. Fatty acid methyl esters of MO and chicken liver lipids were prepared and run to obtain the retention times for three fatty acids (20:5n3, 22:5n6, and 22:5n3) not contained in the external standard. A response factor of 1.0 was used in the calibration table for the fatty acids not contained in the external standard on the basis of analyses of MO and chicken liver lipids. All fatty acid concentrations in tissues were expressed as micrograms per gram of wet tissue. Fatty acid compositions of dietary lipids were expressed as grams per 100 g of fatty acid methyl esters.

A completely randomized statistical design was used to analyze the dietary treatment effects on measured responses. Differences between treatment means were identified by the Student-Neuman-Kuels postpriori test (Steel and Torrie, 1980).

RESULTS

Fatty acid compositions of the dietary lipids are presented in Table II. The analysis showed that as the level of menhaden oil increased so did the amounts of 17:0, 20:5n3, 22:5n3, 22:6n3, and total unsaturates. Total n - 3 fatty acids and the P:S ratios were greater as the MO content increased. As the amount of CF increased in the lipid treatments, the levels of 16:0, 18:1, 18:2n6, total saturates, total n - 6 fatty acids, and the ratios of n - 6:n - 3 rose compared with the lipid treatments containing greater levels of MO.

The GLC analyses revealed that some of the dietary lipids and tissues contained more than one isomer of 18:1 fatty acids. Positional isomers of 18:1 monoenes were probably present in CF and MO. Most isomers of 18:1 fatty acids had retention times that followed 18:1n9, which were 11.37 and 11.25 min for 18:1 and 18:1n9, respectively. Since the various isomers of 18:1 could not be characterized by the present GLC technique, we report total 18:1 fatty acids.

Table III. Body and Organ Weights and Lipid Measurements in 45-Day-Old Broilers Fed Varying Combinations of Chicken Fat/Menhaden Oil

measurement	chicken fat/menhaden oil						pooled SD
	100:0	80:20	60:40	40:60	20:80	0:100	
body weight, g	1693	1803	1782	1610	1629	1579	210.5
liver weight, g/100 g of body weight	2.4	2.6	2.5	3.0	2.5	2.6	0.36
total liver lipid, mg/10 g	685	608	935	838	785	816	238.7
liver triacylglycerols, $\mu\text{mol/g}$	26	20	37	56	53	41	21.0
heart weight, mg/100 g of body weight	434	451	443	466	430	456	51.9
fat pad weight, mg/100 g of body weight	1044	861	907	682	828	920	272.4
total plasma triacylglycerols, $\mu\text{mol/mL}$	1.04 ^a	0.82 ^{a,b}	0.83 ^{a,b}	0.81 ^{a,b}	0.58 ^b	0.55 ^b	0.2
VLDL + LDL triacylglycerols, $\mu\text{mol/mL}$	0.96 ^a	0.75 ^{a,b}	0.72 ^{a,b}	0.72 ^{a,b}	0.53 ^{b,c}	0.43 ^c	0.2

^{a-c} Mean values with pooled standard deviation. Values in rows with different superscripts are significantly different ($P < 0.05$). Body weights, $n = 10, 11, \text{ or } 12$; total liver lipid, $n = 4$; liver triacylglycerols, $n = 3 \text{ or } 4$; all other measurements, $n = 6$.

Table IV. Fatty Acid Compositions (Micrograms per Gram) of Liver Lipids in 45-Day-Old Broilers Fed Varying Combinations of Chicken Fat/Menhaden Oil

fatty acid	chicken fat/menhaden oil						pooled SEM
	100:0	80:20	60:40	40:60	20:80	0:100	
16:0	6514	5262	14028	10766	11210	9280	3023.6
16:1 n 7	861	421	1971	1092	903	773	494.3
17:0	22 ^b	63 ^{a,b}	83 ^{a,b}	82 ^{a,b}	131 ^a	131 ^a	18.9
18:0	4633	4516	7605	6879	8393	7820	1455.4
18:1	5499	3261	13031	7854	6172	5295	3161.7
t18:2 n 6	ND ^f	72	137	8	183	265	78.2
18:2 n 6	3574	3147	3324	2678	3128	2712	336.9
18:3 n 6	11	49	35	ND	51	81	20.5
18:3 n 3	62	55	87	78	120	111	21.3
20:0	ND	8	27	10	39	17	12.8
20:1 n 9	104	80	162	132	153	159	37.3
20:2 n 6	100	126	107	63	92	76	15.1
20:3 n 6	217 ^a	163 ^b	148 ^b	114 ^b	114 ^b	110 ^b	16.7
20:4 n 6	1372 ^a	1076 ^a	634 ^b	505 ^b	572 ^b	470 ^b	103.5
20:5 n 3	77 ^e	865 ^d	1570 ^c	1890 ^c	3295 ^a	2602 ^b	224.8
22:1 n 9	38	61	37	10	34	27	13.0
22:4 n 6	145 ^a	112 ^a	28 ^b	33 ^b	45 ^b	27 ^b	19.7
22:5 n 6	238 ^a	19 ^b	ND	ND	28 ^b	ND	37.4
22:5 n 3	40 ^c	720 ^{b,c}	1138 ^{b,c}	1173 ^{b,c}	2189 ^{a,b}	3301 ^a	393.0
22:6 n 3	259 ^c	1272 ^b	1525 ^b	1620 ^b	2418 ^a	2660 ^a	200.4
total saturates	11170	9849	21744	17738	19773	17248	4413.5
total unsaturates	6095 ^b	7677 ^b	8732 ^b	8163 ^b	12238 ^a	12413 ^a	921.6
total $n - 6$	5656 ^a	4765 ^{a,b}	4413 ^{a,b}	3402 ^b	4216 ^{a,b}	3740 ^b	405.0
total $n - 3$	438 ^c	2912 ^b	4319 ^b	4760 ^b	8022 ^a	8673 ^a	581.9
$n - 6:n - 3$	12.88 ^a	1.65 ^b	1.03 ^{b,c}	0.72 ^c	0.53 ^c	0.43 ^c	0.22
P:S	0.55	0.81	0.56	0.46	0.65	0.85	0.11

^{a-c} Mean values of four tissues per treatment with the pooled standard error of the means. Values in rows with different superscripts are significantly different ($P < 0.05$). ^f ND = not detected.

Chromatograms from lipid sources containing MO and tissues from chickens fed MO demonstrated an unknown peak (retention time of 14.2 min) immediately following the 18:3 n 3 peak (retention time of 13.6 min). This peak represented approximately 3% of the total area, but was not included in the analyses since its identity could not be confirmed. Menhaden oil is known to contain between 2.1% and 2.8% 18:4 n 3 (Ackman, 1982; Joseph, 1985). A GLC analysis of the same MO source in a previous experiment demonstrated the presence of an unknown peak presumed to be 18:4 n 3 (Phetteplace and Watkins, 1989).

Dietary lipid treatments did not significantly influence body weights, liver, heart, and fat pad weights, total liver lipids, or liver triacylglycerols (Table III). As the amount of dietary $n - 3$ fatty acids increased, the total triacylglycerol levels in plasma and VLDL + LDL tended to decrease (Table III). Feeding MO at 40 g/kg of diet (CF: MO combination of 20:80 w/w) significantly reduced chicken plasma triacylglycerol levels compared with CF at 50 g/kg diet.

Liver tissue of chickens fed increasing levels of MO contained more 17:0, 20:5 n 3, 22:5 n 3, and 22:6 n 3, but the amounts of 20:3 n 6, 20:4 n 6, 22:4 n 6, and 22:5 n 6 decreased

(Table IV). The chickens fed diets with more MO had greater amounts of total unsaturates and total $n - 3$ fatty acids compared with those fed CF. Total $n - 6$ fatty acids and $n - 6:n - 3$ ratios increased in the liver of chickens fed higher levels of CF.

The heart tissue exhibited a fatty acid composition similar to that of liver in chickens fed the same dietary treatments. As the level of MO in the diet increased, the concentrations of 20:5 n 3, 22:5 n 3, 22:6 n 3, and total unsaturates also increased, but amounts of 20:4 n 6 and 22:4 n 6 decreased. The total $n - 3$ fatty acids and the P:S ratios increased, while $n - 6$ fatty acids and $n - 6:n - 3$ ratios decreased with the greater levels of dietary MO (Table V).

As expected, the abdominal fat pads of chickens contained fewer long-chain fatty acids compared with the liver and heart tissues. However, the addition of MO to the diet did result in the accumulation of long-chain $n - 3$ fatty acids (20:5 n 3, 22:5 n 3, and 22:6 n 3) into adipose. There were also increased concentrations of 17:0 and 20:1 n 9 in fat pads of chickens fed the higher levels of MO (Table VI). In contrast, the amounts of 16:0, 18:1, and 18:2 n 6 decreased when MO was fed at the higher levels.

Table V. Fatty Acid Compositions (Micrograms per Gram) of Heart Lipids in 45-Day-Old Broilers Fed Varying Combinations of Chicken Fat/Menhaden Oil

fatty acid	chicken fat/menhaden oil						pooled SEM
	100:0	80:20	60:40	40:60	20:80	0:100	
16:0	2219	2238	2234	3066	2127	1945	366.8
16:1 _{n7}	329	355	280	590	358	307	140.3
17:0	16	28	24	52	40	48	8.6
18:0	1483	1495	1444	1571	1465	1370	81.7
18:1	2714	2542	2311	3268	2054	1797	481.8
t18:2 _{n6}	ND ^f	3	ND	4	ND	ND	1.8
18:2 _{n6}	2468	2414	2087	2388	1855	1588	260.6
18:3 _{n6}	3	7	10	25	26	27	6.6
18:3 _{n3}	42	54	45	84	54	49	20.2
20:0	13	12	14	18	13	17	2.9
20:1 _{n9}	64	61	52	85	63	62	11.5
20:2 _{n6}	62 ^b	85 ^a	59 ^{b,c}	54 ^{b,c}	45 ^{b,c}	39 ^c	4.9
20:3 _{n6}	78 ^{a,b}	93 ^a	89 ^{a,b}	84 ^{a,b}	69 ^b	69 ^b	4.7
20:4 _{n6}	1111 ^a	1024 ^a	909 ^{a,b}	695 ^b	712 ^b	722 ^b	76.5
20:5 _{n3}	26 ^e	404 ^d	776 ^c	1303 ^b	1552 ^{a,b}	1780 ^a	109.3
22:1 _{n9}	6	4	4	3	6	5	4.4
22:4 _{n6}	108 ^a	52 ^b	29 ^{b,c}	28 ^{b,c}	10 ^c	9 ^c	7.8
22:5 _{n6}	30	ND	ND	18	25	23	11.0
22:5 _{n3}	12 ^d	408 ^c	568 ^{b,c}	721 ^{a,b}	728 ^{a,b}	920 ^a	75.2
22:6 _{n3}	ND	171 ^{c,d}	243 ^{b,c}	407 ^{a,b,c}	446 ^{a,b}	521 ^a	66.4
total saturates	3732	3774	3717	4707	3645	3380	448.3
total unsaturates	3942 ^b	4715 ^{a,b}	4815 ^{a,b}	5810 ^a	5522 ^{a,b}	5748 ^a	412.5
total <i>n</i> - 6	3862 ^a	3678 ^a	3183 ^{a,b}	3296 ^{a,b}	2742 ^b	2478 ^b	230.6
total <i>n</i> - 3	80 ^c	1038 ^b	1632 ^b	2514 ^a	2780 ^a	3270 ^a	233.3
<i>n</i> - 6: <i>n</i> - 3	41.02 ^a	3.56 ^b	1.97 ^b	1.34 ^b	1.05 ^b	0.76 ^b	2.86
P:S	1.08 ^c	1.27 ^{b,c}	1.30 ^{b,c}	1.27 ^{b,c}	1.51 ^{a,b}	1.71 ^a	0.07

^{a-e} Mean values of four tissues per treatment with the pooled standard error of the means. Values in rows with different superscripts are significantly different ($P < 0.05$). ^f ND, not detected.

Chickens fed greater amounts of CF had more total saturates, total *n* - 6 fatty acids, and larger *n* - 6:*n* - 3 ratios in abdominal fat pad tissues.

DISCUSSION

The fatty acid compositions of the dietary lipid treatments indicated a gradual change from a saturated to a more polyunsaturated lipid for 100:0 to 0:100 CF:MO, respectively. Chicken fat contains more total saturates, monounsaturates, and total *n* - 6 fatty acids compared with MO (Edwards et al., 1973; Pereira et al., 1976). Menhaden oil was shown to contain more *n* - 3 fatty acids and total polyunsaturates relative to animal fats (Ackman, 1982; Miller et al., 1969).

The *n* - 3 fatty acids in fish oil appear to be the main factor(s) responsible for reduced plasma triacylglycerols in normal (Harris et al., 1984; Sanders and Hochland, 1983) and in hypertriglyceridemic patients (Boberg et al., 1986; Phillipson et al., 1985). Studies in several animal species also indicated that fish oil lowered plasma triacylglycerol levels (Huang et al., 1986; Nalbhone et al., 1989; Wong et al., 1984; Daggy et al., 1987). Huang and co-workers (Huang et al., 1986) found that varying amounts of fish oil depressed plasma triacylglycerols from 1.4 to 1.0 mg/mL in rats. Eicosapentaenoic acid (20:5_{n3}) was shown to decrease triacylglycerol synthesis in cultured rat hepatocytes (Nossen et al., 1986). Mature roosters fed fish oil showed a nonsignificant decrease in plasma triacylglycerols compared with those fed corn oil (Daggy et al., 1987). In the present experiment, the decreased VLDL + LDL triacylglycerol levels appeared to be due to increased dietary *n* - 3 fatty acids, which is in agreement with a previous study using chickens (Phetteplace and Watkins, 1989).

Liver triacylglycerol levels were not affected by the dietary treatments. Apparently, the *n* - 3 fatty acids in the dietary lipids did not affect liver triacylglycerol synthesis even though the triacylglycerol content of the

VLDL + LDL fraction was decreased with increasing dietary MO. Perhaps the liver tissue of chickens fed higher levels of MO had greater rates of fatty acid oxidation. Wong et al. (1984) found that perfused livers from rats fed fish oil had increased fatty acid oxidation. Daggy et al. (1987) suggested that *n* - 3 fatty acids may stimulate the activity of lipoprotein lipase in peripheral tissues, thereby enhancing delivery of triacylglycerol and cholesterol to the extrahepatic tissues while decreasing the amounts being brought back to the liver. Unfortunately, fatty acid oxidation and systemic metabolism of lipids were not evaluated in our experiment.

The fatty acid composition of various chicken tissues is readily modified by dietary lipids (Miller et al., 1967, 1969; Watkins, 1988). Broilers fed a diet containing fish oil showed incorporation of *n* - 3 fatty acids into the liver, heart, and edible portions (Miller and Robisch, 1969; Phetteplace and Watkins, 1989). Feeding cod liver oil was shown to enrich rat heart tissue with *n* - 3 fatty acids (Gudbjarnason and Oskarsdottir, 1977). Rat hearts perfused with eicosapentaenoic acid also demonstrated incorporation of this fatty acid into the cardiac muscle phospholipids (Fragiskos et al., 1986).

In the present experiment, liver and heart tissues from chickens fed the higher levels of MO had lower concentrations of 20:4_{n6}. The reduced levels of 20:4_{n6} might be due to *n* - 3 polyunsaturates inhibiting the synthesis of 20:4_{n6} from linoleic acid (18:2_{n6}), as suggested by Edward and Marion (1963) and Miller et al. (1969), or the lower levels of dietary 18:2_{n6}.

The abdominal fat pads contained more 18:1 (oleate) compared with the liver or heart tissues. Oleate is the predominant fatty acid in adipose tissue of many species including chickens and man (Hilditch and Williams, 1964). The adipose tissue from chickens in this experiment showed incorporation of the long-chain *n* - 3 fatty acids, which is in agreement with Miller et al. (1969) and Miller and Robisch (1969).

Table VI. Fatty Acid Compositions (Micrograms per Gram) of Abdominal Fat Pad Lipids in 45-Day-Old Broilers Fed Varying Combinations of Chicken Fat/Menhaden Oil

fatty acid	chicken fat/menhaden oil						pooled SEM
	100:0	80:20	60:40	40:60	20:80	0:100	
16:0	17887 ^a	17192 ^a	15973 ^{a,b}	14840 ^{b,c}	13041 ^c	13142 ^c	640.1
16:1 _{n7}	6215	6017	5328	5198	5196	5229	298.1
17:0	112 ^f	224 ^e	277 ^d	375 ^c	461 ^b	553 ^a	17.9
18:0	3882	4321	4138	3813	3450	3554	243.8
18:1	29613 ^a	26563 ^b	22197 ^c	17331 ^d	13700 ^e	12262 ^e	856.4
t18:2 _{n6}	73	44	56	60	28	45	16.2
18:2 _{n6}	13893 ^a	14451 ^a	11731 ^b	9030 ^c	8175 ^c	7678 ^c	610.7
18:3 _{n6}	190 ^a	220 ^a	200 ^a	81 ^b	152 ^{a,b}	208 ^a	28.1
18:3 _{n3}	832	947	860	798	843	882	50.8
20:0	11	25	45	39	42	53	11.0
20:1 _{n9}	331 ^d	379 ^{b,c,d}	362 ^{c,d}	418 ^{b,c}	448 ^{a,b}	506 ^a	19.6
20:2 _{n6}	122	119	82	82	77	75	10.7
20:3 _{n6}	91	107	78	62	83	96	11.2
20:4 _{n6}	225	278	224	230	260	284	27.7
20:5 _{n3}	ND ^g	786 ^d	1337 ^d	2210 ^c	2994 ^b	3679 ^a	199.1
22:5 _{n3}	ND	230 ^d	421 ^d	714 ^c	933 ^b	1347 ^a	72.7
22:6 _{n3}	ND	322 ^{c,d}	499 ^{b,c}	696 ^{b,c}	967 ^b	1347 ^a	131.0
total saturates	21892 ^a	21762 ^a	20434 ^a	19067 ^{a,b}	16994 ^b	17302 ^b	839.6
total unsaturates	15428	17503	15486	13963	14513	15668	829.5
total <i>n</i> - 6	14595 ^a	15219 ^a	12371 ^b	9545 ^c	8775 ^c	8386 ^c	652.4
total <i>n</i> - 3	832 ^e	2284 ^d	3116 ^d	4418 ^c	5738 ^b	7282 ^a	333.9
<i>n</i> - 6: <i>n</i> - 3	17.53 ^a	6.82 ^b	3.97 ^c	2.20 ^d	1.54 ^{d,e}	1.16 ^e	0.27
P:S	0.70 ^b	0.80 ^{a,b}	0.76 ^{a,b}	0.73 ^{a,b}	0.86 ^{a,b}	0.91 ^a	0.04

^{a-f} Mean values of four tissues per treatment with the pooled standard error of the means. Values in rows with different superscripts are significantly different ($P < 0.05$). ^g ND, not detected.

The liver, heart, and abdominal fat pad tissues were enriched with *n* - 3 fatty acids similar to the extent of chicken tissues in a previous study (Phetteplace and Watkins, 1989). Although the fatty acid composition of skeletal muscle was not evaluated in the present experiment, these tissues probably contained increased amounts of *n* - 3 fatty acids. Consumption of chicken tissues enriched with *n* - 3 fatty acids would provide a reasonable source of healthful lipids for humans at risk for coronary heart disease. Other possible benefits from the consumption of *n* - 3 fatty acid enriched chicken tissues include reductions in the *n* - 6: *n* - 3 ratio and an increase in the P:S ratio in plasma lipids of hyperlipidemic individuals. Further research is needed to verify that tissues from chickens fed the newer MO products are organoleptically acceptable and can successfully modify lipid metabolism in humans. Previous organoleptic analyses of edible muscle from chickens fed MO have indicated some off-flavors (Miller et al., 1967; Miller and Robisch, 1969). Since the techniques for producing and refining fish oils are much improved, perhaps chicken meat enriched with modern MO would be more acceptable to consumers.

Our results indicate that dietary *n* - 3 fatty acids significantly enriched liver, heart, and adipose tissues in a dose-dependent fashion beginning with 10 g of MO/kg of diet. Feeding MO to chickens tended to lower plasma triacylglycerols, and a significant reduction occurred with the CF:MO combination of 20:80 (MO at 40 g/kg).

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