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Effect of Dietary Fish-Meal on Chicken Serum, Liver and Spleen Fatty Acid Metabolism

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Abstract: Fish meal is the primary component of chicken diets that contributes DHA, therefore it was speculated that increasing dietary fish meal might alter tissue fatty acid metabolism in laying hens. Leghorn chickens (48) were fed diets containing 0, 1.5, 3 or 6% fish meal for 3 and 9 weeks. All fish meal diets (at 3 weeks) produced a significant increase in serum oleic acid (C16:1) compared to control diet. In chickens fed 3% fish meal diets there was a significant increase in serum stearic acid (C18:0) at 3 weeks compared to all other dietary groups. All fish meal diets (at 3 weeks) produced a dose dependent, significant decrease in splenocyte arachidonic acid (AA, C20:4). Furthermore, hens fed 3 and 6% fish meal showed a significant increase in splenocyte nervonic acid (C24:1) compared to control diet-fed hens at 3 weeks. None of the fish meal diets produced any significant changes in liver fatty acids. Overall, poultry diets containing up to 6% by weight fish meal produced tissue specific, modest changes in fatty acids.

Key words: Fish meal, diet, chicken, fatty acids, splenocytes

Introduction

It is well known that the diet plays an important role in altering fatty acid metabolism. Dietary lipids in particular reduce, increase or cause no structural and/or functional change in cells and organ systems depending on the type, amount of lipid used and length of exposure (Yaquoob, 2003, 2004; Stulnig, 2003). Although a substantial amount of work has been reported using fish oil, much less has been published on the consequence of moderate amounts of fish meal on fatty acid metabolism in immune sensitive tissue of poultry. The combination of the type of fatty acids and protein quality in fish meal may be an alternative dietary tool for regulating fatty acid metabolism and structure and function of immune responsive tissues in poultry. Fish meal is one of the major poultry dietary ingredients and is a cheaper, more stable source of long chain n-3 polyunsaturated fatty acid (PUFA) and protein. Most fish meal is derived from either surplus fish unacceptable for human consumption or from fish processing waste material. Approximately 90% of commercial fish meal is made from anchovy, capelin and menhaden and is approximately 60-70% crude protein and contains 3 to 11% long chain PUFA (Hussein and Jordan, 1991). Howe *et al*. (2002) reported that when 10% fish meal from PorcOmega (POM) was included in chicken diets, it increased n-3 fatty acids in chicken meat seven fold. Hill and Smith (1969) observed that there was a greater survival of cockerels infected with *Salmonella gallinarum* when they were fed 10 vs. 40% fish meal. They concluded that 10% dietary fish meal or lower improved survival. Therefore for our initial studies we chose an upper limit of 6% dietary fish meal to produce modest changes in fatty acid metabolism and composition of immune responsive tissues while maintaining the organoleptic quality of the product and health of the hens. This study included varying concentrations of fish meal (0 to 6% by weight) that was fed for up to 9 weeks to determine if fish meal produced compositional changes in fatty acids in sera, splenic mononuclear cells (MNC) and liver.

Materials and Methods

Animals: Forty-eight Single Comb White Leghorn laying hens were housed in individual wire cages and were provided feed and water *ad libitum*. The hens, identified with a numbered leg band, were placed in one of 4 banks of laying cages, with one treatment per bank. The hens were randomly divided into 4 groups, of 12 birds and fed diets containing 0, 1.5%, 3% or 6% fish-meal (Agway, Minneapolis MD). Temperature (66^{O}F ± 5) and humidity (60% ± 10%) were controlled. The hens were maintained on a 14/10 hr light - dark cycle. Animal use was approved by the Center of Veterinary Medicine Animal Care and Use Committee. Six birds from each group were euthanized by CO_2 asphyxiation 3 and 9

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Table 1: Composition of Control and Test Diets

Ingredient	Control	1.5 % FM	3.0 % FM	6 % FM
Ground corn	61.30%	61.30%	61.30%	61.30%
Soy Meal	21.5%	20.0%	18.50%	15.50%
Soy Oil	2%	2%	2%	2%
Protein Blend	5%	5%	5%	5%
Limestone (Ca)`	7%	7%	7%	7%
Alfalfa Meal	2%	2%	2%	2%
Fish Meal	0.00%	1.50%	3%	6%
DL Methione	0.50%	0.50%	0.50%	0.50%
Vitamin/Mineral Mix	0.50%	0.50%	0.50%	0.50%
NaCl	0.30%	0.30%	0.30%	0.30%

Abbreviations: FM, fish meal. The mineral vitamin/mineral mix was obtained from C.S. Akey Inc. Lewisburg, OH.
The Fish meal and grains were obtained from Agway, Minneapolis MN.

Table 2 A: Percent Fat and Protein of Key Ingredient in Fish Meal Diets fed to Laying Chickens

Name	% Fat	% Nitrogen	% Protein
Alfalfa Meal	4.27±0.12	2.50±0.11	15.64±0.67
Cracked Corn	6.06±0.17	1.30±0.13	8.13±0.80
Soy Meal	2.60±0.10	7.31±0.22	45.71±1.37
Fish Meal	10.97±0.22	10.22±0.37	63.94±2.28
Soy Oil	100	ND	ND
Corn Oil	100	ND	ND

Triplicate samples analyzed for % fat and % nitrogen. Data expressed as mean ± sd. % Protein calculated by multiplying % nitrogen by 6.25. ND = not determined

weeks post dietary treatment. Spleens and livers were removed immediately. MNC were isolated from the spleens. Blood was obtained by cardiac puncture from anesthetized birds.

Diets: Composition of diets is presented in Table 1. The vitamins and mineral pre-mix was obtained from C.S. Akey Inc., Lewisburg, OH. Percent fat, nitrogen and protein in the major dietary components and in the total diets are found in Tables 2 A and B, respectively. The fatty acid composition of the diets is found in Table 3.

Isolation of spleen mononuclear cells (MNC): Spleens were washed and minced in HBSS and the residual tissue was removed using a cell strainer. Single cell suspension from the spleens was overlayed onto the Histopaque (density 1.077 g/ml) and centrifuged at 400 x g for 30 min at room temperature. Mononuclear cells obtained from the interface were washed twice with HBSS and suspended in RPMI 1640 complete medium containing 5% heat inactivated fetal calf serum (Life Technologies, Rockville, MD). Cell concentration was adjusted to 8 x 10^6cells/ml.

Lipid extraction, derivatization and analysis: Total lipids were extracted from serum (1 ml), liver (0.5 gm) and splenocytes (~5-7 x 10^7 cells) by the Folch *et al.*, (1957) method using four volumes of chloroform/methanol (2:1) as modified by Wiesenfeld *et al.* (2001). Butylated hydroxytoluelene (1 g/l) was added to prevent lipid oxidation. Fatty acids were transmethylated to form fatty acid methyl esters (FAMES) with methanolic boron trifluoride (Alltech, Deerfield IL). FAMES were analyzed by gas-liquid chromatography (GLC) using a Hewlett Packard 5890 Series II gas chromatograph equipped with flame ionization detector and a 30 m x 0.32 mm Carbowax column (Alltech, Deerfield, IL). The flow rate of helium, the carrier gas, plus the auxiliary gas, hydrogen and air was 32 ml/min. Temperatures for the detector, injector and column were 290, 235 and 210°C respectively. The relative amount of a given fatty acid was directly proportional to the area under the gas chromatography peaks relative to the sum of all the FAMES peaks. Fatty acids were identified by comparison of retention times with authentic fatty acids obtained from NuChek Prep (Elysian MN). The internal standard was heptadecanoic acid (C17:0) (NuChek Prep, Elysian MN).

Statistical analysis: Fatty acid data between the control and different dietary treatments were analyzed by conducting one-way analysis of variance (ANOVA) followed by pairwise multiple comparison procedures (Tukey Test). Analysis System, (SAS/STAT, SAS Institute Inc, Cary, NC) was used. Differences between the treatment groups were considered statistically significant at $P < 0.05$.

Results and Discussion

Control and Test Diets: The hens' diets were isocaloric, isofat (~6%) and isonitrogenous (~20% protein). The amount of soy meal, ranging from 21 to 15%, by weight in the diet was replaced with 0 to 6% fish meal. The dietary fats were derived from soybean oil and meal, cracked corn and fish meal. The most abundant fatty acid in soybean oil and cracked corn is linoleic acid. The amount of fat in fish meal ranges from 3.4 to 11.3% (Hussein and Jordan, 1991; Burns *et al.*, 2003), which agreed with our results of 10.9%. The fatty acid composition of fish oil varies depending on species and

Table 2 B: Percent Fat and Protein in Fish Meal Diets fed to Laying Chickens

Name	% Fish Meal	% Fat	% Nitrogen	% Protein
Diet 1 - Control	0%	6.18±0.17	3.45±0.28	21.50±0.68
Diet 2	1.5%	6.98±0.14	3.08±0.15	19.21±0.93
Diet 3	3.0%	6.38±0.13	3.04±0.48	20.68±2.98
Diet 4	6.0%	6.24±0.14	3.05±0.33	20.28±2.05

Triplicate samples analyzed for % fat and % nitrogen. Data expressed as mean ± sd. % Protein calculated by multiplying % nitrogen by 6.25.

Table 3: Fatty Acid Profiles of Chicken Diets fed Differing Amounts of Fish Meal

Fatty Acid	Diet 1	Diet 2	Diet 3	Diet 4
	0% FM	1.5% FM	3.0 % FM	6 % FM
C12:0	0.30	0.73	0.45	1.19
C14:0	0.48	0.11	0.39	0.43
C15:0	1.40	0.49	0.94	1.02
C16:0	17.33	17.79	16.37	18.18
C16:1	0.56	1.04	0.72	1.64
C18:0	5.64	5.39	5.09	5.49
C18:1	27.28	28.16	26.91	27.38
C18:1t	1.25	1.18	1.24	1.36
C18:2 n-6	37.59	40.64	41.82	36.97
C18:3 n-6	3.76	2.31	2.75	2.35
C20:1	0.89	0.67	0.59	0.65
C20:2 n-6	0.23	0.37	0.39	0.43
C22:0	0.54	0.49	0.45	0.44
C22:1 n-9	0.34	0.36	0.38	0.46
C22:6 n-3	0.17	0.32	0.40	0.52
Total SFA	26.25	25.89	24.53	27.60
Total MUFA	30.59	31.71	30.07	31.79
Total PUFA	41.75	43.64	45.36	40.26
P/S Ratio	1.57	1.69	1.85	1.46
n3/n6	0.41	0.73	0.89	1.29

Abbreviations. FM = Fish Meal; SFA = Saturated Fatty Acids (no double bond); MUFA = Monounsaturated Fatty acids (one double bond); PUFA = Polyunsaturated Fatty Acids (two or more double bonds); P/S = PUFA/SFA ratio; n-3/n-6 = omega 3/omega 6 fatty acid ratio. Note: fatty acids representing less than 0.5% of the total and which did not change between dietary treatments were eliminated from the table and include: C18:3 n-3; C20:0; C24:0; C24:1, C20:5, n-3.

time of year collected (Cahu *et al.*, 2004). The most abundant fatty acid in fish meal derived from menhaden fish is palmitic acid (C16:0) ~16%. Eicosapentaenoic acid (C20:5, n-3, EPA) and docosahexaenoic acid (C22:6, n-3, DHA) are about 3.5 and 4.9 % of total fatty acids (Burns *et al.*, 2003). Our fish meal contained 1.9 and 4.0% EPA and DHA, respectively.

The crude protein content of fish meal is reported to range between 60 and 72% (Hussein and Jordan, 1991) which agrees with our results (~64%). Fish meal is rich in essential amino acids, particularly lysine and sulfur-containing amino acids such as cystine and methionine. Poste (1990) found that an unpleasant fishy flavor increased as the % fish meal (4 to 12%) was increased in chicken diets. Thus an upper limit of 6% fish meal in the diets was chosen as sufficiently high enough to reasonably alter fatty acid metabolism, without decreasing the product quality (taste/smell) or performance. At the levels and times tested the fish meal diets did not alter egg production. Food consumption, and body weight were unaffected by dietary treatments up to 9 weeks. Each chicken produced an average of 34 to 36 eggs during the 9 week feeding period (data not included).

Fish meal diets and splenocyte fatty acids: The effect of fish meal diets on fatty acid profiles of splenic MNC are found in Table 4. The most abundant fatty acid in splenocytes was oleic acid (C18:1, ~30%), followed by palmitic acid (C16:0, ~22%), linoleic acid (C18:2, ~17%) and stearic acid (C18:0, ~11%). There was a dose related significant decrease in splenocyte arachidonic acid (C20:4, AA) at the 3, but not at 9 weeks. In the control diet-fed group, AA was 7.3% of the total splenocyte fatty acids compared to 1.6% in the 6% fish meal-fed group. Fritsche *et al.* (1991) fed 1-day old chicks 7% menhaden oil for 4 weeks and reported a decrease in AA. De novo arachidonic acid is formed from the desaturation and elongation of linoleic acid (C18:2) to form γ-linolenic acid (C18:3), followed by elongation to C20:3 (eicosatrienoic acid) and then desaturation to form C20:4. The fish meal diets appear to produce changes in this pathway (reduction in C18:2 and a decrease in C20:4) at 3 weeks that returns to homeostatic balance by 9 weeks. A decrease in tissue AA has also been observed in birds fed flaxseed and fish oil which also resulted in a decrease in prostaglandin E_2 (Liu and Denbow, 2001; Olomu and Baracos, 1991). This in turn could decrease inflammatory cellular response.

There was an increase in splenocyte nervonic acid content (C24:1, n-9) at 3 weeks, in the 3% and 6% fish meal-fed hens which did not occur in the serum or liver. Nervonic acid is derived from elongation of oleic acid (C18:1), eicosenoic acid (C20:1), docosaenoic acid (C22:1) to form nervonic acid (C24:1). There may have been an induction of fatty acid microsomal biosynthesis of nervonic acid in the spleen at 3 weeks followed by a feed-back inhibition or saturation at 9 weeks. The dietary and physiological factors which control the nervonic acid

Table 4: Effect of Fish Meal Diets on Spleen Cell Fatty Acid Profiles

Fatty Acid	Diet 1		Diet 2		Diet 3		Diet 4	
	3 wks	9 wks	3 wks	9 wks	3 wks	9 wks	3 wks	9 wks
C16:0	22.74±0.79	22.42±1.06	22.74±1.35	23.74±3.48	22.83±0.2.22	22.38±1.03	24.13±2.44	22.34± 0.57
C16:1	1.59±0.54	2.02±0.49	1.71±0.55	2.38±0.39	1.55±0.74	1.91± 0.36	1.54±0.40	1.92± 0.35
C18:0	12.01±2.86	10.28±2.12	10.90±2.52	11.43±2.53	11.95±3.31	10.98±2.85	14.91±3.87	10.34± 2.21
C18:1	30.65±5.33	32.77±4.73	32.89±3.64	27.31±10.66	29.91±4.77	29.91± 4.39	27.47±2.08	32.89±3.60
C18:2 n-6	16.25±4.29	20.36±1.67	17.73±3.25	21.89±5.24	16.03±3.93	19.97± 5.43	15.25±3.96	19.92± 3.31
C20:4 n-6	7.33^{a}± 2.64	5.08±2.93	4.54^{b}±1.84	6.20±3.26	3.30^{b} ±2.44	6.54 ±3.93	1.60^{b}±0.53	5.01±3.19
C24:1n-6	1.47^{b}±0.72	0.67±0.68	0.90^{b}±0.40	1.00±0.12	3.59^{a}±1.40	1.43±0.55	3.16^{a}±1.42	0.95± 0.61
C22:6 n-3	1.54±1.14	0.70±0.29	0.91±0.37	1.46±1.14	1.11±0.55	1.35±0.52	1.05 ±0.60	0.95±0.60
PUFA	28.53	29.20	25.27	32.73	22.58	30.65	23.24	28.42
P/S ratio	0.81	0.76	0.75	0.79	0.67	0.78	0.54	0.75
n3/n6 ratio	0.08	0.05	0.04	0.08	0.08	0.07	0.08	0.06

P / S ratio = Polyunsaturated (PUFA) / Saturated fatty acids (SFA) or (*n*-6 + *n*-3)/ (C16:0 + C18:0)
n-3/n6 = Omega 3 / Omega 6 fatty acid ratio. N = 6/group (gp). At 3 weeks, ^{a,b,c} mean ± sd values in the same row with unlike superscript letters were significantly different ($p < 0.05$). The following fatty acids were detected, are present in small amounts and/or were not statistically different are deleted from the table: C18:1(t);C18:3 (n-3);C20:2 (n-6);C20:3 (n-6).

Table 5: Serum Fatty Acids of Chickens Fed Fish Meal Diets

	Diet 1 - Control		Diet 2 - 1.5 % FM		Diet 3 - 3 % FM		Diet 4 - 6% FM	
Fatty Acids	3 wks	9 wks	3 wks	9 wks	3 wks	9 wks	3 wks	8 wks
C16:0	23.69 ±0.02	26.66±0.84	25.69±0.02	27.15±1.03	26.21±0.20	25.94±0.77	25.87±0.02	25.03±1.16
C16:1c, n-9	0.83^{b}±1.52	2.33±0.38	2.06^{a}±1.19	2.88±0.404	2.02^{a}±2.63	2.10±0.31	1.98^{a}±2.51	2.34±0.40
C18:0	10.32^{b} ±0.02	11.24±2.58	10.18^{b}±0.04	9.73±0.58	12.88^{a}±0.051	9.69±0.62	10.46^{b}±0.008	9.66±0.79
C18:1	41.19 ±0.04	37.62±3.37	38.70±0.07	38.55±1.50	33.72±1.06	39.80±1.91	39.47±0.076	38.43±2.57
C18:2 n-6	12.62 ±0.05	15.48±1.82	15.39±0.01	15.79±1.07	13.14±0.42	15.54±1.75	13.75±0.15	15.17±1.75
C20:4 n-6	2.24 ±0.07	2.11±0.45	1.71±0.06	1.52±0.12	1.99±0.28	2.10±0.21	1.56±0.055	1.92±0.38
C22:6 n-3	0.88±0.30	0.83±0.16	0.94±0.48	1.07±0.13	1.03±1.37	1.24±0.10	1.48±0.431	1.64±0.29
PUFA	14.34	17.19	18.92	19.40	16.42	19.72	16.35	19.85
P/S ratio	0.39	0.42	0.50	0.50	0.39	0.47	0.43	0.53
n3/n6	0.67	0.95	0.79	1.04	1.33	1.00 0.88	1.06	

P / S ratio = Polyunsaturated (PUFA) / Saturated fatty acids (SFA) ratio. n-3 / n-6 = Omega 3 / Omega 6 fatty acid ratio. At 3 weeks ^{a,b,c} mean ± sd (N=6/gp) values in the same row with unlike superscript letters were significantly different ($p<0.05$). The following fatty acids were detected, are present in small amounts and/or were not statistically different are deleted from the table:C14:0; C18:1(t); C18:3 (n-3); C20:2 (n-6); and C20:3 (n-6).

content of specific chicken tissues are not known at this point. Nervonic acid is thought to serve as a second messenger for various cellular processes and mediate the actions of TNF-α and other cytokines in cell injury (Beresewicz *et al.*, 2002). The mechanisms by which these changes might alter immune tissue function include: protein activation, disruption of lipid rafts, by signal transduction, and activation of nuclear receptors and gene expression (Stulnig, 2003). Taken together these changes may help explain the increased phytohemagglutinin (PHA) induced proliferation and CD4 cell population in the 3% fish meal fed hens compared to control diet-fed hens (Babu *et al.*, 2005). Structural changes in splenocyte membrane may precede or lead the observed functional changes at a later date.

Fish meal diets and serum and liver fatty acids: Fatty acid profiles from serum and liver were also examined in our study. The most abundant serum and liver fatty acid was oleic acid (C18:1, ~39%), followed by palmitic acid (C16:0, ~25%), linoleic acid (C18:2, ~15%) and stearic acid (C18:0, ~12%) (Tables 5 and 6), respectively. Palmitic acid can undergo desaturation and/or elongation. There was a significant increase in serum palmitoleic acid (C16:1) in all fish meal diet groups at 3 weeks compared to control diet-fed chickens. It is possible that as fish meal increased in the diet, palmitic acid (C16:0) underwent desaturation (-2H) resulting in an increase in serum palmitoleic acid. It is also likely that some of the palmitic acid underwent elongation causing a significant increase in stearic acid (C18:0) in the 3% fish meal diet group at 3 weeks. The changes did not increase further with dose (6%) or time (9 weeks). This can be explained by the fact that fatty acid desaturation and elongation may have reached a saturation point or triggered feed back inhibition. As the fish meal increased in the diet, there was a non-significant increase in serum DHA (from 0.8% to 1.6%) and as observed in the spleen, a decrease in AA (from 2.2% to 1.6%). A similar result was obtained when cows were fed 5.1% fish meal for 35 days where serum DHA

Table 6: Liver Fatty Acids of Chickens Fed Fish Meal Diets

Fatty Acids	Diet 1 – Control		Diet 2 – 1.5% FM		Diet 3 - 3% FM		Diet 4 - 6 % FM	
	3 wks	9 wks	3 wks	9 wks	3 wks	9 wks	3 wks	9 wks
C16:0	24.90±1.46	24.66±3.24	25.41± 1.61	26.33 ± 0.83	29.97 ± 8.31	24.87±1.65	25.11± 1.3	24.16±1.37
C16:1, n-9	2.13± 0.48	2.32±0.29	2.44± 0.31	2.82± 0.64	2.42± 0.57	2.06±0.51	2.41± 0.49	2.27± 0.46
C18:0	12.12±0.48	11.35±5.07	12.21± 0.85	10.97±1.25	10.35± 4.64	11.70±1.23	12.37±1.9	11.61±1.16
C18:1, n-9	42.04±1.21	34.17±6.25	40.14± 6.75	35.74± 4.95	39.77±7.10	37.64±5.91	40.75± 5.14	37.18± 5.95
C18:2 n-6	11.52±0.37	13.20±1.90	13.80± 4.50	13.30±1.87	11.83± 2.56	12.42±2.15	11.42± 2.40	13.03± 2.37
C20:4 n-6	3.27±1.84	4.13±1.77	3.21±1.80	2.83±1.41	2.71±1.62	2.95±1.63	2.35±1.58	2.86±1.38
C22:6 n-3	1.46±1.14	1.23±0.43	1.61± 0.94	1.29± 0.62	0.76± 0.63	1.41±0.74	1.71±1.03	1.82 ±0.93
PUFA	14.81	15.97	15.02	16.23	14.16	15.41	14.91	16.78
P/S ratio	0.36	0.39	0.42	0.40	0.33	0.39	0.37	0.43
n-3/n-6 ratio	0.13	0.08	0.13	0.10	0.09	0.12	0.16	0.15

P / S ratio = Polyunsaturated (PUFA) / Saturated fatty acids (SFA) ratio. *n*-3 /*n*-6 = Omega 3 / Omega 6 fatty acid ratio. Data represent mean ± sd (N= 6/group). The following fatty acids were detected, are present in small amounts and/or were not statistically different are deleted from the table: C14:0; C18:1(t); C18:2t n-6; C18:3 (n-3); C20:2 (n-6); C20:3 (n-6).

and EPA increased over time from 0.2 up to 0.5% and 1.8 up to 2% respectively, while AA decreased over the first 21 days (Burns *et al.*, 2003).

There were no significant changes in liver fatty acids associated with fish meal diets fed up to 9 weeks. There was a non-significant decrease in liver AA in chickens fed the 3 and 6% fish meal diets compared to controls. There was a non-significant increase in liver DHA in chickens fed 3 and 6% dietary fish meal diets at the 9 week time point. Similar to our observations, White Leghorn hens fed 10% fish meal diets did not significantly alter liver lipids compared to corn based diets (Cherry and Jones, 1982). Navarro *et al.*, (1972) found that even at the 30% fish meal there was only a slight increase in DHA and EPA (3.1 to 4.1% and 0.2 to 0.4% respectively) in chickens and a slight reduction in AA (2.6 to 1.1%) in eggs.

Conclusions: There were time, dose and tissue specific differences in fatty acid metabolism associated with feeding chickens fish meal diets. Overall, our observations suggest that varying the concentration of fish meal (0 to 6% by weight) produced modest, but important changes in fatty acid metabolism. A reduction in spleenocyte arachidonic acid and increase in nervonic acid may help explain the change in chicken MNCs function observed in hens fed fish meal diets (Babu *et al.*, 2005). These results provide additional information to the poultry industry in optimizing dietary fish meal.

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