

Lipids

Essential Fatty Acid Nutrition of the American Alligator (*Alligator mississippiensis*)¹

MARK A. STATON,² HARDY M. EDWARDS, JR., I. L. BRISBIN, JR.,* TED JOANEN** AND LARRY MCNEASE**

Department of Poultry Science, University of Georgia, Athens, GA 30602; *Savannah River Ecology Laboratory, Aiken, SD 29801; and **Rockefeller Wildlife Refuge, Grand Chenier, LA 70643

ABSTRACT The essential fatty acid (EFA) nutrition of young American alligators (*Alligator mississippiensis*) was examined by feeding a variety of fats/oils with potential EFA activity. Over a 12-wk period, alligators fed diets containing 2.5 or 5.0% chicken liver oil grew longer and heavier and converted feed to body mass more efficiently than alligators fed other fat/oil combinations that lacked or contained only trace amounts of arachidonic acid [20:4(*n*-6)]. Alligators fed an EFA-deficient diet (containing only coconut fat as the dietary fat) were the slowest-growing animals and converted feed to body mass least efficiently. However, over a 41-wk feeding period, alligators fed this diet showed no obvious external signs of deficiency other than being reduced in size and unthrifty. Fatty acid composition of heart, liver, muscle, skin and adipose tissue lipids was influenced markedly by dietary fat composition. Tissues varied significantly in response to dietary fat composition. Heart lipids contained the lowest levels of short- and medium-chain fatty acids and the highest levels of arachidonic acid. Arachidonic acid levels were less influenced by diet than were levels of other 20- and 22-carbon polyunsaturated fatty acids. Radiotracer studies indicated that linoleic acid was converted to arachidonic acid in the liver. Nevertheless, tissue arachidonic acid levels also appeared to be maintained by concentration from dietary sources and selective conservation. It appears that a dietary source of arachidonic acid may be required for a maximum rate of growth. *J. Nutr.* 120:674-685, 1990.

INDEXING KEY WORDS:

- alligator • fatty acid nutrition
- essential fatty acid • arachidonic acid

The essential fatty acid requirements of vertebrate species are influenced by a variety of dietary and environmental factors. Among warm-blooded vertebrates, this is perhaps best illustrated by the dietary essentiality of arachidonic acid [20:4(*n*-6)] for cats, which are true

carnivores (1-6). This fatty acid, an essential metabolite and an integral component of phospholipids in a variety of animal tissues (7), is synthesized from linoleic acid [18:2(*n*-6)], which undergoes a two-carbon elongation interspersed between two desaturation steps (8). Most mammalian and avian species presumably carry on this conversion at a rate that is adequate to support optimal body growth and function. In felids, however, tissues other than testes (1) show extremely limited or no synthesis of arachidonic acid (2-6). Consequently, arachidonic acid is a required nutrient for cats (9, 10). Deficiency signs include occasional inflammatory skin lesions, reduced blood platelet aggregation and reproductive failure (1, 2, 11, 12). This nutritional feature of cats apparently evolved in response to the abundant supply of arachidonic acid in regularly consumed tissues of natural prey.

The essential fatty acid (EFA) requirements of fish, which are more complex than those of mammals, are influenced by the temperature and/or salinity of the environment. In general, the EFA requirement for cold-water species is a greater percentage of diet than that for warmwater species (13). Rainbow trout (*Salmo gairdnerii*), typical of coldwater species, are characterized by an (*n*-3) fatty acid requirement that can be satisfied by linolenic acid but not (*n*-6) fatty acids (14, 15). In contrast, the tropical species *Tilapia zilli* requires linoleic or arachidonic acid (16).

It is believed that the (*n*-3) structure, which allows

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²Present address: Mainland Holdings Crocodile Farm, Niugini Table Birds, Pty. Ltd., P.O. Box 962, Lae, Papua New Guinea.

for one more double bond than does the (n-6) structure in a carbon chain of the same length, is advantageous to coldwater species because it facilitates membrane flexibility and permeability at low temperatures (17). Some species, such as the eel, *Anguilla japonica*, require both linoleic and linolenic acids (18). However, neither of these fatty acids will satisfy the EFA requirements of a number of marine fish, which require (n-3) polyunsaturated fatty acids for optimum performance (19). Both red sea bream (*Mylio macrocephalus*) and turbot (*Scophthalmus maximus*) have been shown to convert little oleic acid [18:1(n-9)] to eicosatrienoic acid [20:3(n-9)], linoleic acid to arachidonic acid, or linolenic acid to (n-3) pentaenes or hexaenes (20-22). The reason for the reduction in such conversions is not known. However, red sea bream, turbot and several other fish species reviewed by Watanabe (19) are carnivorous and may therefore have responded to high dietary levels of derived EFAs in the same way that cats have responded to dietary arachidonic acid. The EFA deficiency signs of fish include reduced growth, poor feed conversion, elevated water content in muscles, caudal fin erosion and swollen, pale livers (17, 19).

American alligators (*Alligator mississippiensis*) are cold-blooded carnivores that inhabit both fresh and brackish, usually warm, aquatic environments. However, during winter they spend much of several months submerged in cold, occasionally icy, water. Because of the variety of EFA requirements among vertebrates that can be associated with a carnivorous nature, environmental temperature and the salinity of the environment, the EFA requirements of alligators are not easily predictable. Knowledge of alligator EFA requirements is of practical interest in the formulation of feeds for farmed alligators. Currently, the optimum diet for farmed alligators is considered to be the carcass of nutria (*Myocastor coypu*), a large marsh-dwelling rodent trapped for its fur. Analysis of the carcass fat of this species has provided no clues regarding alligator EFA requirements because it has been found to contain considerable quantities of linoleic (17.8% of fatty acids), linolenic (14.5%), arachidonic (2.2%) docosapentaenoic [22:5(n-3); 1.0%] and docosahexaenoic (1.0%) acids (23, 24). In the only consideration of crocodilian EFA requirements in the literature, Garnett (25) suggested that eicosapentaenoic acid and docosahexaenoic acid are essential nutrients for the saltwater crocodile (*Crocodylus porosus*). Garnett attributed this to the species' marine ancestry and to its marine and brackish habitat. Here we report on studies of the EFA nutrition of young alligators grown in fresh water at temperatures typical of those for farmed alligators. Data are presented on the performance and fatty acid composition of alligators fed a wide spectrum of potential EFAs, as well as the results of radiotracer studies to assess alligator desaturation-elongation capacities.

TABLE 1
Composition of experimental diets

Ingredient	Amount
	%
Casein	35.50
Isolated soybean protein ¹	26.70
High solubility corn dextrin ²	15.00
Gelatin	2.00
Glycine	2.00
Carboxymethyl cellulose ²	2.00
Limestone	1.50
Defluorinated phosphate	1.50
Potassium carbonate	0.40
Sodium chloride	0.75
Vitamin premix ³	0.40
Magnesium carbonate	0.35
DL-Methionine	0.30
L-Tryptophan	0.20
Trace mineral premix ⁴	0.20
Chromic oxide	0.10
Selenium premix ⁵	0.05
Basal diet total	90.00
Added fat ⁶	10.00

¹ICN Biochemicals, Cleveland, OH.

²Sigma Chemical, St. Louis, MO.

³Provided the following in mg/kg of diet except as noted: vitamin A as all-trans-retinyl acetate, 18,000 IU; cholecalciferol, 2000 IU; vitamin E (all-rac- α -tocopheryl acetate), 150 IU; menadione (as menadione sodium bisulfite), 25; thiamin (as thiamin mononitrate), 15; riboflavin, 15; vitamin B-6, 25; vitamin B-12, 0.042; nicotinic acid, 200; calcium pantothenate, 50; folic acid, 4.0; biotin, 1.0; choline Cl, 1500; inositol, 50; para-amino-benzoic acid, 50; ascorbic acid, 450; ethoxyquin, 150.

⁴Provided the following in mg/kg of diet: Mn (as MnO₂), 240; Zn (as ZnO), 200; Fe (as FeSO₄·7H₂O), 120; Cu (as CuSO₄), 20; I, 4.2; and Ca, 300-360 [as Ca(IO₃)₂ and limestone].

⁵Provided 0.1 mg Se/kg of diet as sodium selenite.

⁶Containing one or more of the following: coconut fat, olive oil, safflower oil, linseed oil, chicken liver oil, fish oil and lard.

METHODS

Animals and housing. Hatchling alligators from four clutches (a clutch being a female alligator's annual, synchronous egg output) were obtained from Rockefeller Wildlife Refuge (Grand Chenier, LA) after being artificially incubated according to the methods of Joanen and McNease (26). Alligators were immediately transported to the University of Georgia facilities by automobile. Four alligators were housed in 0.6 m × 0.6 m opaque plastic tanks filled to a water depth of 8 cm. Water temperature was allowed to fluctuate with room temperature, which varied from 29 to 32°C. A 10 cm × 20 cm plastic platform elevated above water level served both as a dry area for animals to emerge from water and as a feeding station. Tanks were washed and refilled with warm water (27-30°C) after each feeding. The

TABLE 2
Fat and fatty acid composition of experimental diets

	Basal	Coconut fat	Olive oil	Safflower oil	Linseed oil	Chicken liver oil	Fish oil	Chicken liver/fish oil	Mixed oil
	% of diet								
Fat/oil									
Coconut	—	10.0	5.0	5.0	5.0	5.0	5.0	5.0	—
Olive	—	—	5.0	2.0	2.0	—	—	—	—
Safflower	—	—	—	3.0	—	—	—	—	1.5
Linseed	—	—	—	—	3.0	—	—	—	2.0
Chicken liver	—	—	—	—	—	5.0	—	2.5	—
Fish	—	—	—	—	—	—	5.0	2.5	2.5
Lard	—	—	—	—	—	—	—	—	4.0
	% of fatty acids ¹								
Fatty acid²									
8:0	—	4.8	2.1	0.8	0.3	2.4	1.6	1.4	—
10:0	0.1	5.4	2.2	2.3	2.0	3.2	2.8	2.7	0.1
12:0	1.5	49.1	21.4	25.1	24.6	29.8	27.3	27.1	0.5
14:0	2.1	18.9	8.4	9.7	9.7	11.4	16.4	13.4	3.2
14:1	0.1	—	—	—	—	—	0.6	0.4	0.3
15:0	0.1	—	—	—	—	—	0.1	0.1	0.1
16:0	12.6	9.4	11.3	9.5	9.4	14.8	15.0	15.2	17.2
16:1	0.8	—	0.9	0.4	0.4	1.5	7.4	4.9	4.7
16:2	—	—	0.1	tr ³	tr	tr	1.0	0.6	0.7
17:0	—	—	tr	tr	tr	tr	0.5	0.3	0.6
18:0	5.1	5.6	5.6	6.0	6.5	11.4	8.4	9.9	8.3
18:1	61.4	4.6	39.3	19.0	21.9	13.8	7.1	10.7	27.2
18:2	12.1	0.7	8.4	27.0	8.7	7.0	1.0	5.0	21.3
18:3	1.0	—	0.9	0.7	16.3	0.3	1.2	0.9	11.1
20:4	—	—	—	—	—	2.7	0.4	1.3	0.2
20:5	0.2	tr	tr	tr	tr	0.4	5.8	3.0	2.4
22:5	—	—	—	—	—	0.1	0.3	0.2	0.1
22:6	—	—	—	—	—	0.3	1.6	1.3	0.9
Other C20,22 ⁴	—	—	tr	—	—	0.4	1.2	1.4	1.1

¹By analysis.

²Carbon chain length:number of double bonds.

³Traces, < 0.05%.

⁴20- and 22-carbon fatty acids, including 20:0, 20:1, 22:0, 22:1 and 22:4.

photoperiod was maintained on a 14L:10D schedule. Six weeks after the animals' arrival at the housing facilities, the feeding study was initiated. Prior to this, alligators were fed ground lean pork, supplemented with vitamins (as noted in Table 1) and limestone (3% of dietary dry matter).

Experimental diets. Eight diets fed to alligators were identical except for added fat, which was included at 10% of the diet (Table 1, Table 2). Prior to addition of fat, the diet contained 0.4% total lipids, which contributed 0.05% linoleic and 0.004% linolenic acid to the finished diets. The coconut fat diet was considered to be EFA deficient because it contained little or no linoleic or linolenic acid (Table 2). The olive oil diet provided linoleic acid at 0.84% of diet, whereas the safflower oil diet provided abundant supplies (2.7% of diet) of this fatty acid. The linseed oil diet provided linoleic and

linolenic acids at 0.87 and 1.63% of diet, respectively. Chicken liver oil, extracted using a chloroform/methanol procedure for extraction of fat from large amounts of tissue (27), was fed in the chicken liver oil diet, which supplied the known parent or derived EFAs of the (*n*-6) family, including an abundant supply of arachidonic acid (0.27% of diet). Similarly, fish oil (Standard Products, Kilmarnack, VA) supplied parent and derived (*n*-3) EFAs in the fish oil diet. The fat of the chicken liver/fish oil diet, which contained all EFAs of the (*n*-3) and (*n*-6) families, was made by including both chicken liver and fish oil in equal amounts. A mixture of 25% fish oil, 40% lard, 15% safflower oil and 20% linseed oil was included in the mixed oil diet. This mixture of fat was similar in fatty acid chain length and degree of unsaturation to the carcass fat of nutria. The mixed oil diet apparently contained adequate amounts of those fatty

TABLE 3

Alligator average total lengths, gains in body weight, dry matter feed consumption and efficiency, liver size and liver lipids as a function of dietary treatment and clutch¹

	Weeks 1-12						
	Weeks 5-12		Total length	Body weight gain	Liver		
	Dry matter consumption	Feed efficiency ²			Weight	%Body weight	Total lipids
	g	g/g	cm	g	g	g/g	%
Diet							
Coconut fat	123 ^d	0.95 ^c	49.0 ^c	168 ^c	4.47 ^d	1.76 ^c	2.72
Olive oil	181 ^c	1.13 ^b	50.9 ^b	264 ^b	7.25 ^{bc}	2.08 ^a	3.08
Safflower oil	192 ^{bc}	1.12 ^b	51.3 ^b	274 ^b	6.59 ^c	1.83 ^{bc}	3.59
Linseed oil	189 ^{bc}	1.12 ^b	51.2 ^b	278 ^b	7.20 ^{bc}	1.98 ^{ab}	2.89
Chicken liver oil	220 ^a	1.25 ^a	53.8 ^a	341 ^a	8.14 ^{ab}	1.98 ^{ab}	3.03
Fish oil	187 ^{bc}	1.12 ^b	50.6 ^b	270 ^b	7.42 ^{bc}	1.93 ^{abc}	3.91
Chicken liver/fish oil	219 ^a	1.25 ^a	53.6 ^a	343 ^a	8.79 ^a	2.09 ^a	3.50
Mixed oil	206 ^{ab}	1.14 ^b	51.8 ^b	297 ^b	6.89 ^{bc}	1.82 ^{bc}	4.37
P	0.001	0.001	0.001	0.001	0.001	0.002	0.188
Clutch							
1	203 ^a	1.27 ^a	52.8 ^b	329 ^a	8.55 ^a	2.10 ^a	3.08 ^b
2	217 ^a	1.14 ^b	53.8 ^a	323 ^a	8.07 ^a	1.96 ^b	4.15 ^a
3	178 ^b	1.06 ^c	50.3 ^c	252 ^b	6.71 ^b	2.00 ^{ab}	2.76 ^b
4	159 ^c	1.07 ^c	47.8 ^d	213 ^c	5.13 ^c	1.65 ^c	3.61 ^{ab}
P	0.001	0.001	0.001	0.001	0.001	0.001	0.023
X	189	1.13	51.3	279	7.18	1.94	3.40
SEM ³	7	0.03	0.51	11	0.39	0.06	0.42

¹Column means, for diet or clutch, sharing the same superscript letter are not significantly different. Differences were established using Duncan's new multiple range test when analysis of variance indicated significant differences ($p \leq 0.05$).

²Live body weight gain/dry matter feed consumption.

³Pooled standard error of the mean from the analysis of variance.

acids known to be essential for other species, except for arachidonic acid, which constituted only 0.02% of finished dietary dry matter. This diet was the only diet that did not contain at least 5% coconut fat.

To feed the diets, water was added (40% by weight) to form a moistened cake, which was stored at -20°C for up to 1 mo. Prior to feeding, feed was warmed to room temperature and chopped into bite-sized (~0.5 cm³) pieces. Feed was offered three times weekly for 1-2 h. Animals were judged to have reached satiation because some feed remained at the end of each feeding. Dry matter consumption was calculated by difference using the daily recorded weight of feed offered and uneaten and adjusting for moisture.

Feeding study. At 6 wk of age, alligators were segregated according to clutch and size, such that there were eight tanks of four alligators of equal average weight for each of the four clutches. One tank from each group of eight was randomly assigned to each dietary treatment. Thus, each dietary treatment contained one replicate (tank of four alligators) from each clutch. The feeding study was begun (d 1) by feeding alligators a mixture of

lean pork and the moistened experimental diet (60/40 by wet weight). The animals were weaned onto the experimental diets by including the pork in decreasing amounts until, after 2 wk, no pork was included in the diet. Animals from each pen were weighed (± 0.5 g) collectively at the initiation of the experiment and after 4 and 12 wk of feeding. Feed consumption was recorded and feed efficiency calculated only for wk 4-12. Total length was measured (± 0.5 mm) individually at the end of the study. After the final weighing, one animal from each replicate was killed by cervical dislocation, frozen and stored at -20°C. Upon thawing, the liver, heart, samples of muscle and adipose tissue from the ventral basal region of the tail, and soft skin from the side of the body were removed for determination of total lipid content and fatty acid composition as described below.

To observe for potential EFA deficiency signs, the remaining animals from the coconut fat dietary treatment were fed this experimental diet for an additional 29 wk (total of 41 wk). After a total of 27 wk, four alligators were killed, frozen and stored prior to dissection as described previously. Six individuals from the

TABLE 4
Average fatty acid composition of adipose tissue from alligators fed diets differing only in fat composition¹

Fatty acid ²	Diet									p	Fatty
	Coconut oil	Olive oil	Safflower oil	Linseed oil	Chicken liver oil	Fish oil	Chicken liver/fish oil	Mixed oil	Standard error ³		
	% of fatty acids										
10:0	0.8 ^{ab}	0.8 ^{abc}	0.7 ^c	0.7 ^{bc}	0.9 ^a	0.9 ^a	0.9 ^{ab}	0.1 ^{ab}	0.05	0.001	10:0
12:0	18.2 ^a	12.0 ^d	11.2 ^d	12.6 ^{cd}	15.8 ^b	12.4 ^{cd}	13.5 ^c	1.1 ^c	0.48	0.001	12:0
14:0	9.7 ^a	6.3 ^e	6.0 ^e	6.3 ^e	7.2 ^d	8.6 ^b	8.0 ^c	2.7 ^f	0.15	0.001	14:0
14:1	0.7 ^a	0.3 ^c	0.4 ^{bc}	0.3 ^c	0.2 ^c	0.6 ^{ab}	0.4 ^{bc}	0.5 ^{bc}	0.08	0.001	14:1
15:0	0.3	0.1	0.1	tr ^a	0.5	0.1	0.1	0.2	0.20	0.581	15:0
16:0	16.9 ^b	15.4 ^c	14.7 ^c	15.1 ^c	18.3 ^a	18.3 ^a	18.3 ^a	18.0 ^a	0.28	0.001	16:0
16:1	10.0 ^b	6.6 ^e	6.7 ^e	6.5 ^e	7.6 ^d	10.8 ^a	8.4 ^c	8.2 ^{cd}	0.22	0.001	16:1
16:2	1.2 ^a	0.6 ^{bc}	0.9 ^b	0.6 ^b	0.5 ^b	1.2 ^{ab}	0.9 ^{ab}	1.3 ^a	0.11	0.005	16:2
17:0	0.9 ^a	0.5 ^b	0.7 ^{ab}	0.5 ^{bc}	0.5 ^c	0.7 ^b	0.8 ^b	0.9 ^a	0.11	0.001	17:0
18:0	3.0 ^e	3.8 ^c	3.2 ^{de}	3.4 ^{cde}	5.2 ^a	4.3 ^b	5.1 ^a	3.5 ^{cd}	0.17	0.001	18:0
18:1	26.9 ^g	41.9 ^a	30.7 ^{cd}	32.3 ^c	30.3 ^{de}	26.5 ^e	28.6 ^{cd}	35.1 ^b	0.66	0.001	18:1
18:2	9.9 ^d	9.3 ^d	23.4 ^a	11.8 ^c	10.2 ^d	7.5 ^e	8.1 ^c	19.6 ^b	0.41	0.001	18:2
18:3	1.5 ^c	1.5 ^c	1.8 ^c	10.5 ^a	1.4 ^c	1.8 ^c	1.8 ^c	7.6 ^b	0.35	0.001	18:3
20:0	— ^b	0.1 ^b	— ^b	— ^b	— ^b	tr ^b	0.2 ^a	— ^b	0.03	0.002	20:0
20:3	— ^b	0.1 ^a	— ^b	— ^b	tr ^b	tr ^b	tr ^b	— ^b	0.01	0.001	20:3
20:4	0.3 ^b	0.5 ^b	0.4 ^b	0.4 ^b	1.2 ^a	0.6 ^b	1.1 ^a	0.4 ^b	0.12	0.001	20:4
20:5	0.1 ^c	0.2 ^c	tr ^c	0.2 ^c	0.2 ^c	3.0 ^a	1.6 ^b	1.3 ^b	0.15	0.001	20:5
22:4	0.1 ^{bc}	0.1 ^{bc}	tr ^c	tr ^c	tr ^{bc}	0.1 ^{ab}	0.2 ^a	tr ^{bc}	0.03	0.002	22:4
22:5	tr ^b	0.1 ^b	0.1 ^b	0.1 ^b	tr ^b	0.5 ^a	0.5 ^a	0.1 ^b	0.08	0.001	22:5
22:6	0.1 ^a	0.2 ^a	0.1 ^a	0.1 ^a	0.3 ^a	1.7 ^b	1.8 ^b	0.5 ^a	0.14	0.001	22:6
Other C20,22 ⁵	—	0.4	0.4	0.2	0.1	0.7	0.6	0.8	0.24	0.104	Other
24:1	0.1	0.1	tr	tr	tr	0.1	0.1	tr	0.03	0.098	24:1

¹Values in a row that do not have a common superscript letter are significantly different ($p \leq 0.05$) as assessed by ANOVA and Duncan's new multiple range test.

²Carbon chain length:number of double bonds.

³Pooled standard error of the mean.

⁴Traces, < 0.05%.

⁵20- and 22-carbon fatty acids, including 20:1, 22:0 and 22:1.

olive oil group were fed the olive oil diet after the feeding trial. Two of these alligators and one fed the coconut fat diet were randomly selected after a total of 13–25 wk for studying the conversion of linoleic acid to arachidonic acid.

Analytical methods. Carcass, tissue and feed lipids were extracted using the method of Folch et al. (28). Fatty acids from the Folch extract were methylated by refluxing in 5% sulfuric acid in methanol (v/v) for 2.5 h. Following extraction with petroleum ether, fatty acid composition was determined by gas-liquid chromatography using column conditions and methods of identification described elsewhere (29).

Synthesis of arachidonic acid from linoleic acid. [1-¹⁴C]Linoleic acid (Amersham, Arlington Heights, IL) was purified by preparative thin layer chromatography (27), transferred into olive oil and quantified in a Packard Instruments (Downers Grove, IL) Model 3255 Tri-Carb liquid scintillation spectrometer. Alligators were administered 1.67 to 4.60 μ Ci of [1-¹⁴C]linoleic acid in 1–2

mL of olive oil directly into the stomach by pipette. The alligators were then housed in tanks under conditions described previously and were observed for several hours to ensure that they did not regurgitate the oil. After 48 h, they were killed by decapitation, and their livers were removed, immediately frozen and stored at -20°C . After thawing, liver lipids were extracted (28) and three aliquots taken. One was counted for ¹⁴C activity. Another aliquot was separated into neutral lipid and phospholipid fractions using a silicic acid column (27) to determine the relative composition and ¹⁴C activity of these lipid fractions. The third aliquot was methylated, as described previously. Methyl esters were separated according to the number of double bonds using argentation thin layer chromatography (27). Recovery of ¹⁴C-labeled fatty acids in the tetraene band was considered evidence of conversion of the administered [¹⁴C]linoleic acid to arachidonic acid.

Statistical analyses. Differences between responses were determined using the *t*-test or general linear model.

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TABLE 5

Average fatty acid composition of heart tissue from alligators fed diets differing only in fat composition¹

p	Fatty acid ²	Diet								Standard error ³	p
		Coconut oil	Olive oil	Safflower oil	Linseed oil	Chicken liver oil	Fish oil	Chicken liver/fish oil	Mixed oil		
		% of fatty acids									
.001	10:0	0.2	0.2	0.5	0.4	0.5	0.2	0.3	0.1	0.25	0.810
.001	12:0	2.8	3.7	6.4	5.5	5.2	1.6	1.8	0.4	2.09	0.436
.001	14:0	3.3 ^a	2.9 ^{ab}	3.8 ^a	3.2 ^{ab}	3.1 ^{ab}	2.6 ^{ab}	2.4 ^{ab}	1.0 ^b	0.76	0.194
.001	14:1	0.1 ^{ab}	0.2 ^{ab}	0.4 ^{ab}	0.5 ^a	0.1 ^{ab}	0.3 ^{ab}	tr ^b	— ^b	0.30	0.193
.581	15:0	1.4 ^{bc}	0.8 ^d	1.1 ^{cd}	0.6 ^d	0.8 ^{cd}	2.2 ^a	2.0 ^a	1.8 ^{ab}	0.22	0.001
.001	16:0	13.2 ^b	13.3 ^{ab}	13.7 ^{ab}	13.5 ^{ab}	14.7 ^a	14.6 ^{ab}	14.4 ^{ab}	13.2 ^b	0.49	0.089
.001	16:1	5.9 ^a	4.5 ^{ab}	4.4 ^{ab}	4.3 ^{ab}	5.3 ^a	4.9 ^a	4.1 ^{ab}	2.9 ^b	0.76	0.055
.005	16:2	0.8 ^{bc}	0.6 ^c	0.5 ^c	0.4 ^c	0.6 ^c	1.5 ^a	1.5 ^a	1.0 ^b	0.19	0.001
.001	17:0	0.1 ^b	0.4 ^{ab}	0.4 ^{ab}	0.4 ^b	0.2 ^b	0.7 ^a	0.3 ^b	0.2 ^b	0.11	0.042
.001	18:0	15.1 ^a	11.3 ^{ab}	10.2 ^b	10.7 ^{ab}	11.7 ^{ab}	14.9 ^a	14.7 ^{ab}	15.3 ^a	2.74	0.047
.001	18:1	22.3 ^{bc}	30.5 ^a	22.5 ^{bc}	24.4 ^b	21.1 ^{bc}	19.4 ^c	19.9 ^c	21.8 ^b	2.22	0.001
.001	18:2	15.5 ^{bc}	15.7 ^b	22.4 ^a	15.5 ^{bc}	12.6 ^c	7.8 ^d	9.7 ^d	17.8 ^b	0.99	0.001
.001	18:3	1.0 ^c	0.6 ^c	0.8 ^c	6.5 ^a	0.5 ^a	0.8 ^c	0.3 ^c	2.6 ^b	0.51	0.001
.002	20:0	0.3 ^a	0.3 ^{ab}	0.3 ^{ab}	— ^b	— ^b	— ^b	0.2 ^{ab}	0.4 ^a	0.10	0.011
.001	20:3	1.5 ^b	2.3 ^a	0.8 ^{bc}	0.8 ^{bc}	0.9 ^{bc}	0.4 ^c	0.4 ^c	0.4 ^c	0.30	0.001
.001	20:4	10.1 ^{bc}	8.6 ^{bc}	9.6 ^{bc}	6.3 ^c	17.6 ^a	6.3 ^c	12.4 ^b	7.8 ^{bc}	1.89	0.001
.001	20:5	2.8 ^d	1.5 ^d	0.7 ^d	6.8 ^c	2.9 ^d	17.6 ^a	12.0 ^b	9.8 ^b	1.03	0.001
.002	22:4	0.3 ^{cd}	0.5 ^{bc}	0.4 ^{cd}	0.1 ^d	0.4 ^{cd}	0.6 ^{abc}	0.9 ^{ab}	0.9 ^a	0.14	0.001
.001	22:5	0.2 ^c	0.3 ^{bc}	0.5 ^{abc}	0.9 ^{ab}	0.8 ^{abc}	1.1 ^a	0.8 ^{abc}	1.1 ^a	0.22	0.021
.001	22:6	0.4 ^{bc}	0.3 ^{bc}	0.2 ^c	0.3 ^{bc}	1.1 ^{abc}	1.7 ^a	1.3 ^{ab}	1.1 ^{abc}	0.35	0.013
.104	Other C20,22 ⁴	1.8 ^a	0.5 ^{bcd}	0.9 ^b	0.1 ^d	0.3 ^{cd}	0.5 ^{bcd}	0.6 ^{bc}	0.9 ^b	0.20	0.001
.098	24:1	0.9	0.7	0.7	0.2	0.7	0.6	0.9	0.3	0.28	0.401

¹Values in a row that do not have a common superscript letter are significantly different ($p \leq 0.05$) as assessed by ANOVA and Duncan's new multiple range test.

²Carbon chain length:number of double bonds.

³Pooled standard error of the mean.

⁴20- and 22-carbon fatty acids, including 20:1, 22:0 and 22:1.

RESULTS

Feeding study. Alligators fed the coconut fat diet ate less, converted feed to body weight less efficiently, were shorter and gained less body weight than animals fed the other diets (Table 3). Dry matter consumption, feed efficiency, body weight gains and total body length of alligators fed the chicken liver oil and the chicken liver/fish oil diets were significantly greater than for those fed the other diets. There were no significant differences in these responses for the olive oil, safflower oil, linseed oil, fish oil or mixed oil diets. Liver weight varied according to dietary treatment, and this was not

entirely due to differences in body size attained. Expressed as a percentage of body weight, livers were smallest from alligators fed the coconut fat diet, largest from those fed the olive oil and fish oil diets, and intermediate in size for all others. Lipid content of the liver (% of wet weight) did not vary with dietary treatment.

Significant differences in performance criteria and liver weight and lipid content existed among alligators from different clutches (Table 3). Alligators from clutches 1 and 2 ate more, converted feed to body mass more efficiently, gained more weight and length and had heavier livers than those from clutches 3 and 4. Expressed as a percentage of body weight, livers from the slowest-growing clutch-4 alligators were the smallest. Although liver lipids did differ between clutches, there was no obvious relationship between these differences and those in performance criteria or liver size.

Tissue fat composition. As a percentage of wet weight, lipids constituted $69.1 \pm 7.7\%$ (mean \pm SD) of

TABLE 6
Average fatty acid composition of liver tissue from alligators fed diets differing only in fat composition¹

Fatty acid ²	Diet								Standard error ³	p
	Coconut oil	Olive oil	Safflower oil	Linseed oil	Chicken liver oil	Fish oil	Chicken liver/fish oil	Mixed oil		
	% of fatty acids									
10:0	0.9 ^{ab}	0.6 ^b	0.8 ^{ab}	0.8 ^{ab}	0.7 ^{ab}	0.8 ^{ab}	1.1 ^a	0.1 ^c	0.15	0.004
12:0	11.5 ^a	8.2 ^a	9.4 ^a	9.5 ^a	10.3 ^a	10.8 ^a	10.7 ^a	0.5 ^b	1.15	0.001
14:0	8.4 ^a	4.7 ^c	5.0 ^c	4.8 ^c	5.4 ^c	7.1 ^b	6.1 ^{bc}	2.0 ^d	0.44	0.001
14:1	1.5 ^a	0.3 ^b	0.4 ^b	0.3 ^b	0.1 ^b	0.4 ^b	0.4 ^b	0.3 ^b	0.14	0.001
15:0	0.2 ^a	0.1 ^{ab}	0.2 ^a	0.1 ^{ab}	0.1 ^{ab}	0.1 ^{ab}	0.1 ^{ab}	tr ^{ba}	0.04	0.114
16:0	18.0 ^{bc}	16.9 ^c	16.8 ^c	17.4 ^c	19.9 ^a	19.1 ^{ab}	19.4 ^{ab}	20.0 ^a	0.57	0.001
16:1	10.3 ^a	6.7 ^e	5.8 ^f	6.8 ^{ef}	7.4 ^d	9.4 ^b	8.3 ^c	6.8 ^{de}	0.23	0.001
16:2	0.5 ^{bc}	0.3 ^d	0.5 ^{bcd}	0.3 ^d	0.4 ^{cd}	0.7 ^a	0.2 ^{cd}	0.6 ^{ab}	0.06	0.001
17:0	0.4 ^{abc}	0.2 ^{bc}	0.5 ^{ab}	0.3 ^{abc}	0.4 ^{abc}	0.6 ^a	0.2 ^c	0.3 ^{abc}	0.10	0.155
18:0	7.4	5.9	6.1	6.0	7.2	6.5	6.9	6.5	0.71	0.053
18:1	22.4 ^{ef}	37.7 ^a	25.2 ^{cd}	27.9 ^{bc}	26.6 ^{cd}	20.4 ^f	24.8 ^{de}	29.7 ^b	0.95	0.001
18:2	6.9 ^e	8.8 ^{cd}	19.3 ^a	9.5 ^c	8.7 ^d	4.8 ^f	6.0 ^e	15.8 ^b	0.35	0.001
18:3	0.8 ^c	0.6 ^c	0.6 ^c	8.1 ^a	0.6 ^c	1.0 ^c	0.9 ^c	6.2 ^b	0.25	0.001
20:0	0.3 ^{ab}	0.4 ^a	0.3 ^a	—	tr ^c	tr ^c	0.2 ^{bc}	0.3 ^a	0.05	0.001
20:3	1.9 ^a	1.3 ^b	0.8 ^c	0.6 ^{cd}	0.4 ^{cd}	0.2 ^d	0.2 ^d	0.2 ^d	0.18	0.001
20:4	3.8 ^{bc}	4.3 ^{bc}	6.9 ^a	3.2 ^{bc}	8.5 ^a	2.7 ^c	4.9 ^b	3.0 ^{bc}	0.65	0.001
20:5	1.3 ^c	0.8 ^c	0.6 ^a	5.2 ^b	1.4 ^c	9.7 ^a	4.9 ^b	5.2 ^b	0.39	0.001
22:4	0.3 ^{bc}	0.3 ^b	0.5 ^{ab}	0.1 ^c	0.4 ^b	0.5 ^{ab}	0.7 ^a	0.6 ^{ab}	0.19	0.002
22:5	0.4 ^c	0.1 ^d	0.3 ^{cd}	0.4 ^{cd}	0.3 ^{cd}	1.3 ^a	1.9 ^b	0.9 ^b	0.09	0.001
22:6	0.6 ^d	0.3 ^d	0.3 ^d	0.4 ^d	1.7 ^e	3.1 ^a	2.5 ^b	1.7 ^c	0.18	0.001
Other C20,22 ⁵	1.1 ^a	0.4 ^{bcd}	0.5 ^{bc}	0.1 ^d	0.2 ^{cd}	1.2 ^a	0.7 ^b	0.7 ^b	0.12	0.001
24:1	0.5 ^{ab}	0.3 ^{abc}	0.1 ^c	0.1 ^{bc}	0.5 ^a	0.5 ^{ab}	0.2 ^{abc}	0.1 ^c	0.11	0.031

¹Values in a row that do not have a common superscript letter are significantly different ($p \leq 0.05$) as assessed by ANOVA and Duncan's new multiple range test.

²Carbon chain length:number of double bonds.

³Pooled standard error of the mean.

⁴Traces, < 0.05%.

⁵20- and 22-carbon fatty acids, including 20:1, 22:0 and 22:1.

adipose tissue, $3.4 \pm 1.0\%$ of liver, $1.4 \pm 0.5\%$ of muscle, $1.4 \pm 0.6\%$ of heart and $1.0 \pm 0.3\%$ of skin.

Adipose tissue fatty acid composition (Table 4) was highly influenced by diet. Adipose tissue from alligators fed the mixed oil diet, which did not include coconut fat, contained significantly lower levels of lauric and myristic acids than did adipose tissue of alligators fed other diets. Adipose tissue from those fed the coconut fat diet, which included twice as much coconut fat as any other diet, contained higher concentrations of lauric, myristic and myristoleic (14:1) acids but lower levels of most fatty acids with 18 or more carbon atoms than did adipose tissue of alligators fed other diets. Adipose tissue from alligators fed the olive oil, safflower oil and linseed oil diets contained high levels of oleic, linoleic and linolenic acids, respectively. Similarly, the chicken liver oil, fish oil, chicken liver/fish oil and mixed oil diets contained high levels of palmitoleic acid and the long-chain polyunsaturated fatty acids that characterized these diets.

The high levels of arachidonic and eicosapentaenoic acids of heart lipids were influenced by dietary treatment (Table 5). The highest levels were found in heart lipids from alligators fed the chicken liver and fish oil diets, which averaged 17.6% arachidonic acid and 17.6% eicosapentaenoic acid, respectively. Compared with heart lipids from alligators fed the coconut fat, olive oil and safflower oil diets, heart lipids of alligators fed the linseed oil diet contained significantly greater levels of eicosapentaenoic acid. However, when dietary levels of linolenic or eicosapentaenoic acids were low (coconut fat, olive oil, safflower oil and chicken liver oil diets), heart lipid concentrations of this fatty acid were also low (0.7–2.9%). This suggests that desaturation and elongation of dietary linolenic acid was occurring. Thus, eicosapentaenoic acid was not selectively concentrated in heart lipids unless there was a dietary source of the acid or its 18-carbon precursor. In contrast, arachidonic acid was present in heart lipids at levels equal to or greater than 6.3% of fatty acids, regardless of the dietary

TABLE 7

Average fatty acid composition of muscle tissue from alligators fed diets differing only in fat composition¹

p	Fatty acid ²	Diet								Standard error ³	p
		Coconut oil	Olive oil	Safflower oil	Linseed oil	Chicken liver oil	Fish oil	Chicken liver/fish oil	Mixed oil		
		% of fatty acids									
0.004	10:0	0.5 ^{abc}	0.7 ^{ab}	0.6 ^{ab}	0.4 ^{bc}	0.4 ^{bc}	0.9 ^a	0.5 ^{abc}	0.1 ^c	0.18	0.037
0.001	12:0	15.1 ^a	10.4 ^{ab}	8.6 ^b	7.7 ^b	7.2 ^b	11.6 ^{ab}	6.0 ^b	0.4 ^c	1.91	0.001
0.001	14:0	8.2 ^a	5.3 ^b	4.9 ^b	4.6 ^b	4.5 ^b	6.9 ^a	4.8 ^b	1.6 ^c	0.52	0.001
0.001	14:1	0.8	0.3	0.3	0.2	0.2	0.5	0.2	0.9	0.20	0.061
0.114	15:0	1.1 ^b	0.7 ^b	1.2 ^b	0.7 ^b	1.5 ^b	1.0 ^b	2.4 ^a	1.1 ^b	0.33	0.012
0.001	16:0	16.0 ^{cd}	15.3 ^d	15.2 ^d	16.0 ^{cd}	18.4 ^a	16.5 ^{bcd}	18.0 ^{ab}	17.5 ^{abc}	0.64	0.003
0.001	16:1	9.3 ^a	6.6 ^b	5.2 ^c	5.9 ^{bc}	7.1 ^b	8.9 ^a	6.7 ^b	5.3 ^c	0.43	0.001
0.001	16:2	0.8 ^b	0.5 ^b	1.0 ^{ab}	0.6 ^{ab}	0.6 ^{ab}	1.1 ^a	0.5 ^b	0.9 ^{ab}	0.17	0.078
0.155	17:0	0.3 ^b	0.4 ^{ab}	0.3 ^b	0.5 ^{ab}	0.6 ^a	0.6 ^a	0.4 ^{ab}	0.3 ^b	0.10	0.040
0.053	18:0	5.9 ^{cd}	5.2 ^d	6.1 ^{cd}	6.5 ^{bcd}	8.0 ^{ab}	6.6 ^{bcd}	8.9 ^a	7.7 ^{abc}	0.43	0.002
0.001	18:1	25.7 ^c	37.8 ^a	26.1 ^c	30.2 ^b	28.8 ^{bc}	21.8 ^d	25.6 ^c	28.8 ^{bc}	1.37	0.001
0.001	18:2	8.7 ^c	10.6 ^d	20.9 ^a	12.7 ^c	12.4 ^c	6.5 ^f	8.3 ^c	16.1 ^b	0.05	0.001
0.001	18:3	1.9 ^{cd}	0.7 ^d	2.0 ^c	8.2 ^a	0.7 ^d	1.0 ^{cd}	1.3 ^{cd}	5.5 ^b	0.44	0.001
0.001	20:0	0.2	0.5	0.3	—	0.2	—	0.2	0.3	0.13	0.090
0.001	20:3	0.4 ^{abc}	0.6 ^a	0.4 ^{abc}	0.1 ^a	0.5 ^{ab}	0.1 ^{cd}	0.3 ^{bcd}	0.2 ^{cd}	0.10	0.002
0.001	20:4	1.8 ^c	1.9 ^c	3.6 ^b	2.0 ^c	5.5 ^a	1.9 ^c	3.7 ^b	2.9 ^{bc}	0.42	0.001
0.001	20:5	0.7 ^c	0.6 ^c	0.6 ^c	2.0 ^c	1.2 ^c	7.9 ^a	5.7 ^b	4.8 ^b	0.58	0.001
0.002	22:4	0.2 ^{cd}	0.3 ^c	0.5 ^b	0.1 ^d	0.3 ^b	0.3 ^c	0.8 ^a	0.6 ^b	0.69	0.001
0.001	22:5	0.5 ^b	0.2 ^b	0.5 ^b	0.7 ^b	0.4 ^b	1.4 ^a	1.4 ^a	1.6 ^a	0.51	0.007
0.001	22:6	1.2 ^c	0.7 ^c	0.9 ^c	0.7 ^c	1.5 ^c	2.7 ^{ab}	3.3 ^a	2.3 ^b	0.29	0.001
0.001	Other C20,22 ⁴	1.6	1.2	1.2	0.7	0.7	1.1	1.3	1.2	0.36	0.800
0.031	24:1	0.2	0.2	0.1	0.1	0.4	0.2	0.2	0.2	0.22	0.391

¹Values in a row that do not have a common superscript letter are significantly different ($p \leq 0.05$) as assessed by ANOVA and Duncan's new multiple range test.
²Carbon chain length:number of double bonds.
³Pooled standard error of the mean.
⁴20- and 22-carbon fatty acids, including 20:1, 22:0 and 22:1.

availability of this fatty acid or its 18-carbon precursor. That this level may have been maintained by tenacious conservation of the fatty acid is indicated by its relatively high levels in heart lipids from alligators fed the coconut fat diet. Heart lipids from alligators fed coconut fat and olive oil diets contained significantly higher levels of eicosatrienoic acid, presumed to be 20:3(n-9) derived from oleic acid. The presence of this triene is yet another indication of the desaturation and elongation capacities required in synthesis of arachidonic acid. The significantly greater levels of stearic acid found in heart lipids varied slightly with diet, but there was no relationship between these levels and the performance supported by the diets. Eighteen-carbon monenes, dienes and trienes in heart lipids were reflective of diet because their levels were greatest with the olive oil, safflower oil and linseed oil diets, respectively.

The liver lipids of alligators (Table 6) fed diets containing high levels of 18-carbon unsaturated fatty acids contained correspondingly elevated levels of the fatty

acids derived from their desaturation and elongation. Thus, liver lipids in alligators fed the linseed oil diet contained eicosapentaenoic acid levels that were significantly greater than those in alligators fed diets other than those containing fish oil. Similarly, liver lipids in alligators fed the safflower oil diet contained arachidonic acid levels that were significantly greater than those in animals fed any diet except the chicken liver oil diet. With regard to desaturation and elongation capacities, it is notable that liver lipid levels of arachidonic acid were higher with the safflower oil diet than with the chicken liver/fish oil diet. The significantly higher levels of eicosatrienoic acid in the liver lipids of alligators fed the coconut fat and olive oil diets are presumed to indicate desaturation and elongation of 18:1(n-9).

In muscle and skin lipids (Tables 7, 8), arachidonic acid levels were highest for the chicken liver oil diet. Arachidonic acid levels in muscle and skin lipids of alligators fed the safflower oil and chicken liver/fish oil diets were statistically similar and were greater than

TABLE 8
Average fatty acid composition of skin tissue from alligators fed diets differing only in fat composition¹

Fatty acid ²	Diet									p
	Coconut oil	Olive oil	Safflower oil	Linseed oil	Chicken liver oil	Fish oil	Chicken liver/fish oil	Mixed oil	Standard error ³	
	% of fatty acids									
10:0	0.1	0.4	0.1	0.2	0.5	0.4	0.7	0.1	0.18	0.149
12:0	10.9	6.3	3.4	7.3	6.7	4.6	4.6	4.3	1.54	0.436
14:0	5.2	4.7	4.5	5.0	5.7	5.5	5.3	5.3	0.76	0.916
14:1	1.1 ^a	0.3 ^b	0.4 ^b	0.3 ^b	0.4 ^b	0.5 ^b	0.5 ^b	0.3 ^b	0.16	0.037
15:0	0.5 ^{bc}	0.4 ^c	0.7 ^{abc}	0.5 ^{bc}	0.5 ^{bc}	1.1 ^{ab}	1.2 ^a	0.7 ^{abc}	0.21	0.055
16:0	16.7 ^a	15.5 ^b	16.1 ^{ab}	15.1 ^b	16.8 ^{ab}	16.8 ^{ab}	16.1 ^{ab}	17.5 ^a	0.65	0.134
16:1	7.5	6.2	5.8	6.2	7.5	7.7	6.5	7.3	0.58	0.093
16:2	0.5 ^c	0.5 ^c	0.6 ^c	0.7 ^{bc}	0.7 ^{bc}	1.3 ^a	1.6 ^a	0.9 ^{bc}	0.23	0.012
17:0	1.4	0.5	0.8	0.7	0.3	0.8	0.7	0.5	0.25	0.499
18:0	6.9	5.6	6.6	6.3	6.7	6.7	7.4	5.5	0.63	0.335
18:1	24.4 ^{bc}	31.7 ^a	22.6 ^{bc}	25.5 ^b	23.0 ^{bc}	21.7 ^{bc}	21.0 ^c	24.1 ^{bc}	1.43	0.001
18:2	13.3 ^{bc}	11.3 ^{cd}	17.7 ^a	12.0 ^c	9.7 ^{de}	6.4 ^f	7.7 ^{ed}	14.9 ^b	0.80	0.001
18:3	1.5 ^{cd}	0.7 ^d	3.1 ^{bc}	9.5 ^a	1.4 ^{cd}	0.5 ^d	1.1 ^d	3.7 ^b	0.65	0.001
20:0	3.7 ^{ab}	1.7 ^{bed}	1.0 ^{cd}	— ^d	2.7 ^{abc}	1.6 ^{bcd}	4.5 ^a	2.4 ^{abc}	0.81	0.006
20:3	1.6 ^a	1.4 ^a	0.5 ^b	tr ^{ba}	0.3 ^b	0.2 ^b	0.2 ^b	0.2 ^b	0.18	0.001
20:4	5.4 ^{bc}	4.6 ^c	7.1 ^b	3.9 ^c	11.1 ^a	4.5 ^c	7.2 ^b	3.3 ^c	0.89	0.001
20:5	1.2 ^{de}	0.6 ^e	1.3 ^{de}	2.5 ^{cd}	1.7 ^{de}	9.2 ^a	5.0 ^b	3.7 ^{bc}	0.65	0.001
22:4	0.8 ^b	0.6 ^{bc}	0.8 ^b	0.2 ^c	0.9 ^b	1.1 ^b	2.1 ^a	0.8 ^b	0.21	0.001
22:5	0.4 ^c	0.6 ^c	1.8 ^{abc}	2.9 ^a	0.9 ^{bc}	2.2 ^{ab}	0.8 ^{bc}	0.7 ^{bc}	0.50	0.007
22:6	0.4	0.7 ^{ab}	1.0 ^{ab}	0.5 ^{ab}	0.7 ^{ab}	2.3 ^c	1.2 ^b	0.7 ^{ab}	0.31	0.005
Other C20,22 ⁵	2.3 ^a	1.3 ^{ab}	0.5 ^{bc}	0.6 ^{bc}	0.1 ^c	0.4 ^{bc}	1.4 ^{ab}	1.9 ^a	0.37	0.002
24:1	1.2	0.7	0.4	0.9	0.6	0.7	0.9	0.7	0.25	0.469

¹Values in a row that do not have a common superscript letter are significantly different ($p \leq 0.05$) as assessed by ANOVA and Duncan's new multiple range test.

²Carbon chain length:number of double bonds.

³Pooled standard error of the mean.

⁴Traces, < 0.05%.

⁵20- and 22-carbon fatty acids, including 20:1, 22:0 and 22:1.

levels with the remaining diets. In contrast, eicosapentaenoic acid levels in muscle and skin lipids were elevated only when a dietary source of this fatty acid existed. The significantly greater levels of arachidic acid found in skin, as compared with other tissues, did vary with dietary treatment. There was no apparent relationship between levels of this fatty acid and the alligator performance produced by the respective diets. Levels of arachidic acid were greatest in skin tissue of alligators fed diets containing higher levels of stearic acid, perhaps signifying elongation of stearic acid.

Extended feeding of an essential fatty acid-deficient diet. Alligators fed the coconut fat diet for 27 wk were only slightly heavier and longer than those fed the chicken liver oil and chicken liver/fish oil diets for the initial 12-wk feeding period. The levels of many fatty acids in tissues of alligators fed the coconut fat diet for 27 wk were different from those resulting from 12 wk of feeding (Table 9). Most changes in composition occurred for fatty acids with fewer than 18 carbons. After

27 wk of feeding, significantly higher levels of medium-chain-length fatty acids were observed for adipose tissue, heart and skin. Lauric acid accounted for almost one-fourth of the fatty acids in adipose tissue. Palmitoleic acid levels increased in lipids of all tissues except muscle. Muscle was the only tissue in which the percentage of eicosatrienoic acid (+92%) and arachidonic acid (+24%) increased after the additional 15 wk of feeding the coconut fat diet. However, because total lipids in these tissues decreased by 31%, arachidonic acid, expressed per gram of total muscle, did not increase. The same is true for the increase in oleic acid in muscle lipids. The percentage composition of most other fatty acids with a chain length of 18 or more carbons decreased or was not significantly changed as a result of the additional 15 wk of feeding the coconut fat diet. Where increases did occur, very low levels of fatty acids were involved.

External deficiency signs. Despite a total of up to 41 wk of feeding of the coconut fat diet to six alligators, the

TABLE 9

Fat composition of tissues from alligators fed the essential fatty acid-deficient coconut fat diet for 27 weeks and the percentage change in fatty acid composition relative to alligators fed the same diet for only 12 weeks

	Fat composition ¹						Relative change in composition ²								
	Adipose tissue	Heart	Liver	Muscle	Skin	Adipose tissue	Heart	Liver	Muscle	Skin	Adipose tissue	Heart	Liver	Muscle	Skin
	%						% change								
Total lipids ³	70.4 ± 1.6	1.1 ± 0.2	2.8 ± 0.5	1.2 ± 0.1	0.9 ± 0.2	NSD	NSD	NSD	-31	NSD	NSD	NSD	NSD	NSD	NSD
Fatty acid ⁴															
10:0	0.9 ± 0.04	0.4 ± 0.33	0.3 ± 0.43	0.3 ± 0.20	0.6 ± 0.36	NSD	NSD	-64 ^c	NSD	NSD	NSD	-64 ^c	NSD	NSD	+560 ^c
12:0	23.9 ± 0.32	10.5 ± 4.2	13.2 ± 5.38	0.1 ± 3.2	0.9 ± 2.4	+31 ^a	+273 ^b	NSD	-46 ^a	+303 ^b	+273 ^b	NSD	NSD	-46 ^a	+303 ^b
14:0	13.1 ± 5.0	6.8 ± 1.4	9.6 ± 2.2	7.5 ± 0.96	9.1 ± 0.40	+3 ^a	+105 ^a	NSD	NSD	+73 ^a	+105 ^a	NSD	NSD	NSD	+73 ^a
14:1	1.3 ± 0.47	1.3 ± 0.38	1.9 ± 0.40	1.1 ± 0.24	0.7 ± 0.41	+85	+871 ^a	NSD	NSD	NSD	+871 ^a	NSD	NSD	NSD	NSD
15:0	—	0.9 ± 0.34	0.2 ± 0.18	2.3 ± 0.38	1.0 ± 0.2	-ND ^b	NSD	NSD	+103 ^a	NSD	NSD	NSD	NSD	NSD	NSD
16:0	15.8 ± 0.39	13.0 ± 0.84	18.6 ± 1.7	15.6 ± 0.63	13.9 ± 0.36	-8 ^a	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	-20 ^b
16:1	10.9 ± 0.47	10.0 ± 1.6	14.8 ± 1.5	10.6 ± 1.7	9.9 ± 0.73	+9 ^c	+70 ^a	+44 ^a	NSD	NSD	+70 ^a	+44 ^a	NSD	NSD	+32 ^a
16:2	0.7 ± 0.05	1.0 ± 0.16	0.5 ± 0.11	0.7 ± 0.53	1.0 ± 0.54	-44 ^b	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
17:0	0.3 ± 0.38	0.7 ± 0.46	—	0.3 ± 0.29	1.4 ± 0.26	-66 ^b	+425 ^c	-ND ^c	NSD	NSD	+425 ^c	-ND ^c	NSD	NSD	NSD
18:0	3.6 ± 0.17	10.9 ± 2.1	5.2 ± 0.78	8.2 ± 1.6	5.8 ± 0.21	+22 ^b	-28 ^c	-29 ^a	+38 ^b	NSD	-28 ^c	-29 ^a	+38 ^b	NSD	-16 ^b
18:1	22.5 ± 0.57	21.4 ± 1.7	24.5 ± 3.3	9.6 ± 1.2	10.4 ± 0.92	-16 ^a	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	-25 ^a
18:2	6.2 ± 0.26	12.7 ± 1.8	6.1 ± 0.84	9.6 ± 0.87	10.2 ± 0.53	-37 ^a	-18 ^b	NSD	NSD	NSD	-18 ^b	NSD	NSD	NSD	-23 ^c
18:3	0.9 ± 0.07	0.5 ± 0.31	0.3 ± 0.12	0.6 ± 0.16	0.5 ± 0.10	NSD	-51 ^b	NSD	-217 ^a	NSD	-51 ^b	NSD	-217 ^a	NSD	+17 ^a
20:0	—	0.1 ± 0.20	tr ⁵	0.1 ± 0.21	4.1 ± 0.00	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
20:3	tr	1.5 ± 0.40	1.2 ± 0.42	0.8 ± 0.16	0.9 ± 1.61	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
20:4	0.4 ± 0.02	5.7 ± 1.3	2.1 ± 0.63	2.2 ± 0.27	4.9 ± 0.71	NSD	NSD	-38 ^c	+92 ^b	NSD	NSD	-38 ^c	+92 ^b	NSD	-46 ^a
20:5	0.2 ± 0.03	1.8 ± 0.45	0.6 ± 0.26	0.9 ± 0.21	1.0 ± 0.10	NSD	NSD	-44 ^b	+24 ^b	NSD	NSD	-44 ^b	+24 ^b	NSD	NSD
22:4	tr	0.5 ± 0.07	0.3 ± 0.12	0.2 ± 0.07	1.6 ± 0.30	+100 ^c	-34 ^c	-51 ^b	NSD	NSD	-34 ^c	-51 ^b	NSD	NSD	NSD
22:5	0.1 ± 0.05	0.2 ± 0.05	0.2 ± 0.06	0.4 ± 0.16	0.2 ± 0.03	NSD	+79 ^b	NSD	NSD	NSD	+79 ^b	NSD	NSD	NSD	+47 ^c
22:6	0.3 ± 0.11	0.1 ± 0.03	0.2 ± 0.15	0.9 ± 0.24	0.2 ± 0.41	+197 ^b	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	-55 ^a
Other C20,22 ⁶	0.2 ± 0.20	0.7 ± 0.30	0.7 ± 0.23	1.6 ± 0.41	0.7 ± 0.12	+342 ^b	NSD	-58 ^b	NSD	NSD	NSD	-58 ^b	NSD	NSD	-45 ^a
24:1	tr	0.6 ± 0.18	0.3 ± 0.21	0.2 ± 0.19	0.7 ± 0.11	+ND ^c	-64 ^a	NSD	NSD	NSD	-64 ^a	NSD	NSD	NSD	-69 ^c

¹Values are means ± SD.

²Significant differences determined using Student's *t*-test. Superscripts a, b and c indicate $p < 0.01$, 0.05 and 0.10, respectively; NSD = no significant changes. ND = numerical value of change not determined because of trace or zero levels of a fatty acid. + and - indicate significant increases and decreases, respectively.

³% of wet weight.

⁴% of fatty acids.

⁵Traces, < 0.05%.

⁶Other 20- and 22-carbon fatty acids, including 20:1, 22:0 and 22:1.

only obvious external morphological differences were reduced body size and unthrifty (long and thin) appearance.

Arachidonic acid synthesis. For the four alligators dosed with [1-¹⁴C]linoleic acid, only 1.5–2.5% of radioactivity was recovered in liver lipids. Most (77.2–81.3%) of the radioactivity recovered was present in phospholipids, which accounted for 47–57.1% of liver lipids. The vast majority (89.4%) of the ¹⁴C label recovered in liver lipids was associated with the band containing the diene fraction (Table 10). The tetraene fraction accounted for an average of 5.9% of radioactivity. Slightly less (4.8%) was accounted for in the band containing pentaenes and hexaenes. The observed concentrations of ¹⁴C activity in these bands would not be expected unless some of the administered [1-¹⁴C]linoleic acid had undergone the desaturation and elongation steps necessary to synthesize arachidonic acid from linoleic acid.

DISCUSSION

Results of the feeding study indicated that growth performance and feed efficiency of young alligators were significantly improved by a dietary source of arachidonic acid. This suggests that arachidonic acid may be an essential nutrient for maximum growth in young alligators. Based on the fatty acid compositions of the fish oil and chicken liver/fish oil diets, the presumed dietary arachidonic acid requirement appears to be between 0.04 and 0.13% of diet. A level of 0.27% of diet (in the chicken liver diet) did not result in significant improvements in any of the performance criteria over that achieved with 0.13% dietary arachidonic acid (chicken liver/fish oil diet).

The level of dietary arachidonic acid required could be influenced by the amount of linoleic acid in the diet because the results of these experiments indicate that alligators have the enzyme systems required to synthesize arachidonic acid from linoleic acid. Evidence of this conversion can be found in the greater levels of arachidonic acid in tissue lipids of alligators fed safflower oil, as compared with those fed the olive oil or linseed oil diets; the increased presence of eicosatrienoic acid, presumably 20:3(*n*-9), in tissues of the alligators fed the olive oil diet; and the direct elongation and desaturation of the ¹⁴C-labeled linoleic acid. The amount of ¹⁴C from labeled linoleic acid found in the tetraene fraction of liver lipids of the alligator is more comparable to the conversions in the rat (31) than to the very low conversions observed in the cat (32). Further research is required to investigate the possibility that feeding linoleic acid levels greater than 2.7% of diet, as was present in the safflower oil diet, might reduce or eliminate the influence of dietary arachidonic acid on alligator performance. However, the fact that alligators synthesize some arachidonic acid from linoleic acid is not incon-

TABLE 10

¹⁴C Activity originating in linoleic acid and recovered from argentation thin layer chromatography fractions of liver lipids of alligators fed essential fatty acid-deficient diets.

Fraction by number of double bonds	Radioactivity recovered ¹
	%
0-3	89.4 ± 6.66
4	5.9 ± 3.42
5, 6, origin	4.8 ± 3.30

¹Values are means ± SD; n = 3.

sistent with a dietary essentiality of the derived EFA. For example, eicosapentaenoic acid and docosahexaenoic acid can be synthesized in limited quantities by some fish for which such long-chain high unsaturated (*n*-3) fatty acids are dietetically essentially (22). Kanazawa et al. (22) concluded that the capacity to desaturate and elongate linolenic acid among various aquatic species is roughly associated, in an inverse relationship, to the dietary requirement of that species for the derived EFA.

The function of arachidonic acid in young alligators at this point, is a matter of speculation. In cats, linoleic acid meets the EFA requirements associated with membrane structure, including growth, skin and coat conditions (3, 12). Dietary arachidonic acid is essential in its role as an eicosanoid precursor, and deficiency impinges on eicosanoid functions in platelet aggregation and reproduction (3, 11, 12). Reproductive involvement obviously cannot be implicated in explaining our observations on hatching alligator growth. Eicosanoid functions may be involved but need not be implicated. In view of dietary arachidonic acid's influence on growth of alligators and of the relative abundance and constancy of this fatty acid in lipids of tissues such as the liver, muscle, skin and heart, it seems reasonable to suggest an essential structural role in membranes. Within the membrane bilayer, arachidonic acid could contribute to membrane fluidity and influence a broad spectrum of membrane-based cell functions (33, 34).

We have analyzed (data not reported here) the influence of clutch on fatty acid composition of the various tissues studied. Only 13 of 100 statistical tests were found to be significant. Most differences were associated with adipose tissue and are assumed to be attributable to clutch-related variation in performance.

The relationship between clutch and performance is receiving considerable attention in the field of alligator farming. Interestingly, the association need not be solely a genetic one. The sex and growth rate of hatching alligators are determined by the temperature at which eggs are incubated (35–37). Data presented here indicate

that clutch affects feed consumption and efficiency, as well as growth. Future studies on alligator nutrition should consider interactions between diet and clutch effects.

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