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Effects of Dietary Fatty Acid Ethyl Esters on Polar and Nonpolar Tissue Lipids in Red Drum, Sciaenops Ocellatus.

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EFFECTS OF DIETARY FATTY ACID ETHYL ESTERS ON POLAR AND NONPOLAR TISSUE LIPIDS IN RED DRUM, *Sciaenops ocellatus*

A Dissertation

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ABSTRACT

Red drum juveniles of mean initial weight 9.23 ± 0.27 g were kept in indoor aquaria. The water was maintained at a salinity of 12 ± 0.5 ppt. The fish were fed six isonitrogenous (38% crude protein) and isocaloric (1.36×10^4 kJ/kg), purified diets differing in lipid content, for nine weeks, to investigate the effects of selected dietary essential fatty acids (EFA) on growth performance and tissue fatty acid composition. Each diet, except the low-fat (LF) diet, contained 8% total lipid from (1) menhaden fish oil (MFO), (2) oleate (18:1n-9) supplemented with linoleate (18:2n-6), (3) linolenate (18:3n-3), (4) a mixture of linoleate and linolenate, or (5) docosahexaenoate (22:6n-3). All fatty acids were provided as ethyl esters (FAEE). Fish fed MFO had highest weight gain and feed efficiency. Weight gain and feed efficiency of fish fed the LF diet was intermediate to that of fish fed MFO and FAEEs. Fish fed FAEE diets had the lowest weight gain and feed efficiency. There was no significant difference in weight gain and feed efficiency among fish fed the FAEE diets. Tissue fatty acid composition generally reflected the fatty acid composition of dietary lipid. Levels of saturated fatty acids (SFAs) in tissues were independent of levels of SFA in the diet. However, fish fed LF and FAEE diets had a decreased level of total SFAs in tissue, while fish fed MFO did not. Levels of monoenoic fatty acids were significantly lower in fish fed MFO than in those fed LF and FAEE diets. N-6 PUFAs were depleted in fish fed diets that did not contain 18:2n-6. N-3 PUFAs increased in muscle lipid of fish fed diets containing 18:3n-3. N-3 highly unsaturated fatty acids (HUFAs) were conserved in the

polar lipid fraction of tissues. Results suggest that the poor growth of fish fed FAEE diets was probably due to the poor palatability and unusual fatty acid composition of those diets. The red drum has very limited ability to chain-elongate and desaturate fatty acids, and presumably has EFA requirements for long-chain unsaturated fatty acids, such as eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (20:4n-6), as do other marine species studied to date.

INTRODUCTION

Red drum (*Sciaenops ocellatus*), also known as channel bass or redfish, are found from Massachusetts to Texas but are most abundant in the Gulf of Mexico. Red drum was an important commercial species along the Gulf coast until the commercial fishery was closed in 1981. As a result, interest in red drum aquaculture has increased.

Red drum is well suited for aquaculture due to its ease of production in hatcheries and its tolerance of a wide range of salinities. It can be induced to spawn on demand at any time of the year (Arnold 1988) and the young can be grown to marketable size (1.4 kg) in indoor raceways or in ponds (Lee et al. 1984, Holt et al. 1990) in about 13-14 months. Red drum is currently the only marine food fish species commercially cultured in the United States (Holt 1992).

Red drum do not tolerate low temperature. A fish kill caused by a freeze in 1989 ruined some Louisiana redfish farmers. At present, there are five commercial red drum farms in Louisiana which, collectively, produced less than 45,000 kg of fish in 1993 (Lorio et al. 1993). Most commercial red drum farms are located in Texas. These farms produce less than 450,000 kg of fish per year. Another potential source of farmed red drum is Ecuador, but there are no hatcheries or broodfish operations in Ecuador. Ecuadorian farmers get red drum fry from Texas hatcheries and raise them in converted shrimp ponds. Their product is less expensive than domestically farmed fish, but not always as high in quality, and supply is inconsistent (Raven 1994).

Nonetheless, red drum has potential as a viable aquacultural product in the U.S. if certain biological and technological constraints can be overcome.

Although red drum has been the focus of many studies concerning environmental requirements (Holt et al. 1981, Holt and Arnold 1983, Lee et al. 1984, Pursley and Wolters 1989, Holt et al. 1990, Thomas and Wolters 1992, Gatlin et al. 1992, Wurts and Stickney 1993), nutritional information on the species is far from complete. Knowledge of nutritional requirements is essential to allow development of a cost-effective, pelleted feed especially for red drum. Feed development is important because feed cost generally represents about 50% of production cost in finfish aquaculture. Reduction of feed cost could contribute substantially to the profitability of red drum aquaculture.

The objectives of this study were: (1) investigate the qualitative requirements for essential fatty acids in red drum, (2) characterize the signs of essential fatty acid deficiencies in red drum, and (3) determine the ability of red drum to elongate and desaturate dietary fatty acids.

LITERATURE REVIEW

Jahncke et al. (1989) investigated whether pond-raised red drum could be used as a replacement for wild red drum in the seafood marketing system of the southeastern United States, by comparing the proximate composition, fatty acid profiles, and sensory characteristics of pond-raised and wild red drum. They found that proximate compositions and sensory characteristics were similar for both pond-raised and wild fish. However, fatty acid profiles of pond-raised fish had higher linoleic acid (18:2n-6) and lower docosahexaenoic acid (22:6n-3) concentrations than those of wild fish, indicating that red drum fatty acid composition reflected the fatty acid composition of the diet. The researchers suggested that pond-raised red drum was a low-fat, high-quality fish that should be an acceptable substitute for wild-caught red drum in the southeastern United States.

To aid conservation efforts, Villarreal et al. (1994) evaluated the efficacy of using fatty acid profiles as a forensic tool to distinguish cultured red drum from illegally marketed wild red drum. They identified four fatty acids as diagnostic. Linoleic acid (18:2n-6) was significantly lower in wild red drum than in cultured red drum. Arachidonic acid (20:4n-6), adrenic acid (22:4n-6), and docosapentaenoic acid (22:5n-6) were significantly higher in wild fish than in cultured fish. They concluded that since fish fatty acid profiles reflect their diets, it is feasible to distinguish wild red drum from farm-raised red drum by examining fatty acid profiles.

Early nutritional studies of red drum were limited to food habits in the wild. Boothby and Avault (1971), using stomach analysis, found fish, shrimp, and crabs to be the main food items in the diet of adult red drum. In a food-habit study on juvenile red drum, Bass and Avault (1975) reported that food habits varied with fish size. Red drum less than 15 mm in length ate zooplankton; red drum between 15 and 75 mm ate small bottom-dwelling invertebrates and small fish; and red drum longer than 75 mm ate decapods, crustaceans, and fish.

Due to the growing interest in aquaculture of red drum, nutritional research has increased in recent years. Lin and Arnold (1983) reported a 50% dietary protein requirement for red drum reared in seawater. Daniels and Robinson (1986) studied the protein and energy requirements of red drum reared in brackish water (5-6 ppt salinity). They reported that 35% protein and a dietary energy level of 1.70×10^4 kJ/g were adequate for good growth (measured in terms of survival, weight gain, and feed conversion) in red drum reared at 22-26°C. Combinations of 44% protein and dietary energy levels of 1.54×10^4 and 1.72×10^4 kJ/g were adequate for red drum reared at 26-33°C. Serrano et al. (1992), using lyophilized red drum muscle as the protein source and menhaden oil as the lipid source, reported that 40% protein and 7-10% lipid provided maximum growth in juvenile red drum reared in brackish water (7 ± 1 ppt salinity). Williams and Robinson (1988) studied the growth response of juvenile red drum reared in brackish water (5 - 6 ppt salinity) when fish were fed diets containing various levels of menhaden oil. The study showed that red drum fed diets containing 7.4 to 11.2% dietary lipid had higher weight gain, feed conversion, and survival than

fish fed a diet with more than 15% dietary lipid. Ellis and Reigh (1991) reported that red drum were less efficient than warmwater fish in utilizing carbohydrates as an energy source. Dietary lipid showed greater protein-sparing effect than dietary carbohydrates in red drum. They suggested that formulated diets for red drum might be improved by incorporating higher dietary lipid and lower carbohydrates levels than those provided in most warmwater fish feeds.

Reigh and Ellis (1992) studied the effectiveness of soybean meal as a fish meal substitute in red drum diets by replacing fish protein with soy-protein in 34% crude-protein diets. They found that soybean was unacceptable as a complete replacement for menhaden fish meal in formulated diets for red drum. A 1:1 mixture of soybean and fish protein was the most cost-effective dietary protein ratio tested on fingerling red drum.

Requirements for two of the indispensable amino acids (lysine and methionine) have been reported for red drum. Lysine is one of the most frequently limiting indispensable amino acids in feedstuffs for warmwater species. Brown et al. (1988), using peanut meal and shrimp-head meal as intact protein, recommended that lysine compose 5.7% of dietary protein in formulated red drum diets. Craig and Gatlin (1992), using freeze-dried red drum muscle as the source of intact protein, reported a lysine requirement of 4.43% of dietary protein for juvenile red drum.

Methionine is an indispensable amino acid required for normal growth and metabolic functions. Dietary cystine exhibits a methionine-sparing effect that reduces the need for methionine. Thus, the requirements for total sulfur amino acids can be

met by either methionine alone or the proper mixture of methionine and cystine. Moon and Gatlin (1991) reported that cystine was able to spare approximately 40% of the dietary methionine requirement on an equimolar sulfur basis. The total sulfur amino acid requirement for juvenile red drum was found to be 3.03% of dietary protein (1.06% of diet).

Minerals are important dietary components that are necessary for various physiological processes. Phosphorus and zinc are the only two minerals for which requirements for red drum have been determined. Davis and Robinson (1987) reported that 0.86% dietary phosphorus was needed for maximum tissue mineralization in juvenile red drum. Gatlin et al. (1991) determined 20-25 mg Zn/kg of dry diet was the minimum dietary zinc requirement for juvenile red drum reared in brackish water (6 $\pm$ 1 ppt salinity).

The recent interest in n-3 fatty acids in the human diet has renewed research activity concerning the role of lipids in fish nutrition and the possibility that cultivated fish intended for human consumption could be n-3 enhanced for improved marketability. Lipids are important dietary components that provide concentrated energy and essential fatty acids (EFAs), and that act as carriers for other fat-soluble nutrients such as sterols and vitamins. Because lipids can also be used to spare dietary protein, the lipid content of the diet is an important factor affecting dietary protein and energy balance, and the efficiency of feed conversion. The fatty acid composition of dietary lipid is also an important consideration because fishes require dietary sources of long-chain polyunsaturated fatty acids for maintenance of membrane permeability and

plasticity, and for enzyme activation and prostaglandin production (Lovell 1989, Stickney and Hardy 1989).

Fishes appear to require fatty acids from the n-3, n-6, and n-9 families for proper metabolism and growth. However, fishes are incapable of synthesizing 18:2n-6, 18:3n-3, 20:5n-3, and 22:6n-3 fatty acids, and dietary sources of n-3 and n-6 fatty acids are probably essential for normal growth and survival (Watanabe 1982). The EFA activity of n-3, n-6, and n-9 fatty acids varies among fish species, and within species as temperature and salinity change (Stickney and Hardy 1989).

Freshwater fishes such as rainbow trout (*Salmo gairdneri*) have EFA requirements for the n-3 family of fatty acids (Castell et al. 1972a, 1972b, 1972c). EFA deficiency symptoms in rainbow trout can be prevented with 1% of methyl linolenate (18:3n-3) in the diet (Watanabe et al. 1974a). Watanabe et al. (1974b) judged the requirement for methyl linolenate in rainbow trout to be between 0.83 and 1.66% of the diet. Yu and Sinnhuber (1975) showed in rainbow trout that 18-carbon n-3 and n-6 fatty acids were converted to longer-chain n-3 and n-6 fatty acids *in vivo*. The capability of rainbow trout to elongate and desaturate n-3 fatty acids was confirmed by Owen et al. (1975), who fed the fish [I-^{14}C] 18:3n-3 and found 70% of the radioactivity present in 22:6n-3. Since rainbow trout are capable of converting 18:3n-3 to longer- chain n-3 fatty acids, 18:3n-3 alone can satisfy their EFA requirement (Watanabe et al. 1974b). Takeuchi and Watanabe (1977b) indicated that 20:5n-3 has the same growth enhancing effect in rainbow trout as 22:6n-3, and that both 20:5n-3 and 22:6n-3 have an EFA activity higher than that of 18:3n-3.

Marine fishes such as red sea bream (*Chrysophyrys major*) also have a requirement for n-3 polyunsaturated fatty acids (PUFAs). Yone and Fujii (1975a, 1975b) found that dietary linolenic acid (18:3n-3) was not critically important for red sea bream. Yamada et al. (1980) demonstrated that red sea bream and other marine fishes like black sea bream (*Mylis macrocephalus*), opaleye (*Girella nigricans*), and striped mullet (*Mugil cephalus*) possess the ability to convert 18:3n-3 to the highly unsaturated fatty acids (HUFAs): 22:5n-3 and 22:6n-3. However, the conversion is slow in these fishes as compared with that in rainbow trout and the requirement for 22:6n-3 (an EFA for marine fishes) may not be satisfied.

Warmwater fishes, like channel catfish (*Ictalurus punctatus*), are not as sensitive to fatty acid deficiencies as rainbow trout and other coldwater species. Stickney and Andrews (1971) found that both beef tallow and menhaden oil supported better growth of channel catfish than safflower oil. The studies of Andrews et al. (1978) showed that animal fat was an excellent dietary energy source for catfish reared at optimum temperature (26-30^{0}C). Stickney and Andrews (1972) found no specific fatty acid requirements for channel catfish and reported that high dietary concentrations of either 18:2n-6 or 18:3n-3 led to inferior growth. Earlier, the researchers had suggested that poor growth of channel catfish fed diets supplemented with vegetable oils was due to poor utilization of linoleic acid. However, in 1983, Stickney et al. suggested that the theory was incorrect and that the poor performance of catfish fed vegetable oils was due to excessive levels of dietary linolenic acid rather than linoleic acid (hydrogenated vegetable oils commonly contain several percent of linolenic acid). Satoh et al. (1989a)

examined the effects of dietary linoleic acid, linolenic acid, and n-3 HUFAs on growth of channel catfish and on the fatty acid composition of catfish liver lipid. Their results did not indicate whether 18:2n-6 and 18:3n-3 were dietary essentials for catfish; however, n-3 HUFAs appeared to enhance growth. The fatty acid composition of polar lipid from liver indicated that channel catfish can convert 18:3n-3 to 22:6n-3. Satoh et al. (1989b) indicated that n-3 fatty acids are essential for channel catfish and that the dietary requirements are 1-2% 18:3n-3 or 0.5-0.75% n-3 HUFAs. Santha and Gatlin (1991) recommended that a dietary source of at least 0.5% n-3 fatty acid be supplemented in practical diets for channel catfish to ensure n-3 EFA adequacy.

Other warmwater fishes, like carp (*Cyprinus carpio*) and eel (*Anguilla japonica*), have dietary requirements for both 18:2n-6 and 18:3n-3. Takeuchi and Watanabe (1977a) found that the dietary requirement for linoleic acid and linolenic acid in carp was about 1% for each fatty acid. Takeuchi et al. (1980) reported that the requirement for 18:2n-6 and 18:3n-3 in eel was each about 0.5% of the diet.

Tilapia zillii, a euryhaline species, requires n-6 fatty acids but not n-3 fatty acids. *T. zillii* is also capable of elongating and desaturating 18:2n-6 to 20:4n-6 (Kanazawa et al. 1980). Takeuchi et al. (1983) found that the dietary requirement for 18:2n-6 in *Tilapia nilotica* (=*Oreochromis niloticus*) was about 0.5% of the diet. Reciprocal-cross hybrid striped bass, or sunshine bass (*Morone chrysops* F x *M. saxatilis* M), the most commonly produced hybrid striped bass in the United States, is another euryhaline species. This species was reported to have limited ability to elongate and desaturate fatty acids (18:1n-9, 18:2n-6, and 18:3n-3), and it has a dietary

requirement for long-chain n-3 fatty acids (20:5n-3 and 22:6n-3) (Nematipour and Gatlin 1993a). The minimum requirement for 20:5n-3 and 22:6n-3 in sunshine bass is approximately 1% of diet (Nematipour and Gatlin 1993b).

Lochmann and Gatlin (1993a, 1993b) investigated the EFA requirements for red drum and reported that a semipurified diet containing menhaden oil, which is rich in 20:5n-3 and 22:6n-3, supported maximum growth. They found that the red drum has limited chain-elongation and desaturation ability, and concluded that 20:5n-3 and 22:6n-3 are dietary essential FAs for red drum. The fish required approximately 0.5% (n-3) HUFA in the diet (approximately 7% of dietary lipid) for proper growth and good health.

MATERIALS AND METHODS

Fish

Two-month-old red drum fingerlings (average size 2 g) were obtained from a commercial producer (Redfish Unlimited) in Palacios, TX. In Palacios, red drum larvae were raised indoors over winter and then stocked as fingerlings into outdoor ponds in the spring. Water for the outdoor ponds was pumped from Matagorda Bay (salinity 14 ppt). Fish were fed a commercial trout feed (Rangen EXTR 400 floating trout feed, Rangen, Inc., Buhl, Idaho).

The fish were transported to the Aquaculture Research Laboratoty, Louisiana State University Agricultural Center, Baton Rouge in a trailer equipped with an oxygen supply. Upon arrival, six fish were randomly selected and taken to the LSU School of Veterinary Medicine for examination of parasites. The remaining fish were kept in an 800-l fiberglass holding tank equipped with a biofilter and aerated with airstones. Fish were fed a mixture of commercial trout feed (Zeigler Bros., Inc., Gardners, PA) and a low-fat, purified diet while acclimating to the indoor environment.

Culture System

Feeding trials were conducted at the Aquaculture Research Laboratory in 38-L tanks grouped in blocks of 24. Each tank was covered with 3-mm (1/8"), grey PVC to minimize disturbance of fish. Each 24-tank block was connected to an upflow biofilter (a series of three, 0.2m^3 boxes) containing clam shells and a polyester-fiber

particle filter. The biofilters were seeded with ammonium chloride and sodium nitrite six months prior to the initiation of the experiment. In each block, water was recirculated with two 1/15-hp submersible centrifugal pumps (Little Giant, model 3E-12N, Oklahoma City, OK), with one pump serving 12 tanks. Flow rate was adjusted to 750 ml/min using PVC ball valves located on the water inlet pipe of each tank. Each tank was provided with continuous aeration through airstones connected to a central air compressor. Salinity of tank water was maintained at 12 $\pm$ 0.5 ppt using Fritz synthetic sea salt mixture (Fritz Chemical Company, Dallas, TX). Salinity and water temperatures were checked 2-3 times per week with a conductivity/salinity meter (Orion, model 140, Boston, MA). Dissolved oxygen concentrations were measured daily with a dissolved oxygen meter (Orion, model 840, Boston, MA). Other water quality parameters (total ammonia-nitrogen, nitrite-nitrogen, and pH) were measured with a Hach portable water test kit (Hach Chemical Company, Loveland, CO). Water quality was maintained within an acceptable range for all parameters throughout the experiment. Water temperatures ranged from 25 - 31°C with a mean of 30°C. Dissolved oxygen was maintained in excess of 6.5 mg/L, and ammonia-nitrogen at 0.3 mg/L or less. Nitrite-nitrogen ranged from 0.02 to 0.5 mg/L and pH from 7.5 to 8.0.

Feeding Trials

Trial 1

After acclimation in the holding tank for one week, six fish (mean initial weight 2 g) were randomly assigned to each of 72 tanks (3 blocks). Eight diet treatments with

various lipid sources were randomly assigned to tanks in each block and all treatments were replicated in triplicate blocks.

This feeding trial was unsuccessful due to high fish mortality. Two factors may have contributed to the high mortality: (1) the fish might have been too small to stand the stress of transportation and handling, and (2) it was difficult to hand-cut the feed to a uniformly small size, so some fish might not have gotten enough to eat and might have died of malnutrition or starvation. The trial was terminated after 7 weeks. No analyses were conducted.

Trial 2

Fish that had been held in a holding tank during trial 1 were used in trial 2. During the holding period, the fish were fed commercial trout feed (Ziegler Bros., Inc. Gardners, PA) once daily at a level of 2% body weight. The experimental design was the same as that used in trial 1, except that six diet treatments were used (Table 1).

In trial 2, six fish (average weight 9.23 $\pm$ 0.27 g) were randomly assigned to each of 48 tanks (2 blocks). The six diet treatments (Table 1) were randomly assigned to tanks in each block (complete randomized block design). The feeding trial ran 9 weeks.

Diet Formulation

Purified diets (Table 2), were formulated (Mixit-2, Agricultural Software Consultants, Kingsville, TX) to be isonitrogeneous (38% crude protein) and isocaloric (1.36×10^4 kJ (3250 kcal)/Kg). One percent defatted fish meal (extracted 3 times with hexane) was included in the diets to improve palatability. The digestible energy-to-

Table 1. Lipids used in experimental diets for trial 2.

Diet	Lipid source[1]
LF[2]	None added
N6	7% oleic acid (18:1n-9), 1% linoleic acid (18:2n-6),
N3	7% oleic acid, 1% linolenic acid (18:3n-3),
N6+3	7% oleic acid, 0.5% linoleic acid, 0.5% linolenic acid
H3	7% oleic acid, 1% docosahexaenoic acid (22:6n-3)
MFO	8% menhaden fish oil[3]

[1] Individual fatty acids provided as fatty acid ethyl esters (Nu Chek Prep Inc., Elysian, Minnesota 56028-0295).
[2] Lipid content was analyzed to be ~ 0.15%.
[3] Sigma Chemical Co., St Louis, Missouri 63178.

Table 2. Composition of basal diet

Ingredients	Percent of Diet
Casein[1] (vitamin free)	43.80
Dextrin[1]	38.55
Cellulose[1]	8.62
Gelatin[1]	1.38
Carboxymethylcellulose[1]	1.50
Calcium phosphate, dibasic[2]	3.75
Vitamin C, additional	0.20
Vitamin mix	
water soluble[3]	0.60
fat soluble[4]	0.10
Mineral mix (phosphorus free)[5]	0.50
Fish meal (defatted)	1.00

[1] United States Biochemical Corp.(USB), Cleveland Ohio 44128.

[2] Mallinckrodt Specialty Chemical Co., Paris, Kentucky 40361.

[3] Contains (as mg/kg): vitamin B_{12}, 0.1; biotin, 5.0; choline chloride 4000.0; folic acid, 15.0; inositol, 1000.0; niacin, 150.0; panthothenic acid 150.0; pyridoxine HCl, 50.0; riboflavin, 100.0; thiamin, 15.0; cellulose, 5485.1.

[4] Prepared in corn oil (with 15 g of ethoxyquin added to make up 100 ml); contains (as mg/kg): cholecalciferol (1.0 IU = 0.025 μg), 0.5; vitamin A acetate (>1,000,000 IU/g), 5.0; alpha-tocopherol (1360 IU/g), 100.0; menadione 6.0.

[5] Contains (as mg/kg): $CoC_{12}\bullet 6H_20$, 0.2; $CuCl_2\bullet 2H_20$, 13.0; $FeSO_4\bullet 7H_20$, 14.9; KI, 3.0; $MgSO_4$, 2480.0; $MnSO_4$, 69.0; Na_2MoO_4, 0.1; Na_2SeO_3, 0.2; $ZnSO_4\bullet 7H_2O$, 880.0; cellulose, 1405.5.

protein ratio was 35.74 kJ (8.55kcal) DE/g protein based on the requirement of catfish. Fatty acid ethyl esters and menhaden fish oil were used as lipid sources. Marine fishes usually lack the ability to chain elongate and desaturate fatty acids, and they have a requirement for n-3 highly unsaturated fatty acids. Therefore, the fatty acids 18:1n-9, 18:2n-6, 18:3n-3, and 22:6n-3 were selected for inclusion in the diets to study effects of these fatty acids on growth and tissue fatty acid patterns. Menhaden fish oil was used as the lipid control because red drum have been shown to perform well when fed menhaden oil (Williams and Robinson 1988). Vitamin and mineral premixes (Table 2) were added following recommended levels for warmwater fishes (National Research Council, 1983). Corn oil was used to prepare the fat-soluble vitamin premix. Ethoxyquin was added as an antioxidant in all diets at a level of 150 mg/kg diet. All known nutrient requirements of red drum (Robinson 1991) were satisfied by these formulations.

Diets were prepared by mixing the dry ingredients in a V-mixer for 5-7 minutes. Menhaden oil or fatty acid ethyl esters and an appropriate amount of cold water (about 40% of dry weight) were then added to the dry ingredients and the wet mixture was blended for an additional 5-7 minutes. Diets were pelleted using a 1/2-hp meat grinder (General Slicing, Murfreesboro, TN) with a 3-mm die. After pelleting, the diets were freeze-dried with a Lyph-lock 18-liter freeze dryer (Labconco Corp., Kansas City, MO) for at least 12 hr and stored frozen at -80°C in an ultra-low-temperature freezer (Harris Manufacturing Company, North Billerica, MA). Portions were removed from the freezer as needed and hand-cut to an appropriate pellet size. Ready-to-feed diets were

kept refrigerated until used. Fatty acid composition of the finished diets was determined by gas chromatography/mass spectrometry (Table 3).

Experimental Procedures

Red drum fingerlings were sorted to uniform size before stocking. Prior to the beginning of the feeding trial, the fish underwent a 5-day conditioning period to adjust to a purified diet and standardized experimental conditions. During the conditioning period, the fish were fed a low-fat diet (LF) to satiation. The day before the feeding trial, fish in each tank were weighed as a group. Weights were taken by placing fish in a tared container of water. Upon initiation of diet treatments, fish were fed 4% of wet body weight daily, divided into two equal feedings (0800 and 1600 hr). Fish were group-weighed every two weeks and feed allotments were adjusted accordingly. Feed consumption was measured four times weekly (two mornings and two afternoons). In the first two weeks, only two consumption measurements were taken each week to avoid stressing the fish while they were acclimating to the experimental conditions. Feed consumption was measured 15-30 minutes after feeding. Uneaten feed was siphoned from the tank, oven dried (Barnstead/Thermolyne Corporation, Dubuque, Iowa) at 85^{0}C for 5 hrs and weighed. As feed consumption stabilized, feeding rate was reduced to 3% of body weight per day in the second week and maintained at 3% until the end of the experiment.

Weight gain, feed efficiency, and survival were used as growth response variables. Weight gain [$WG = (W_f - W_i) \times 100/W_i$] was calculated based on the initial average body weight (W_i) and final average body weight (W_f) of fish in each tank.

Table 3. Fatty acid composition of dietary lipids expressed as a percentage of total fatty acids[1,2]

Fatty acid	CD[3]	Dietary treatment LF	N6	N3	N6+3	H3	MFO
C14:0	0.50	1.06	-	-	-	-	8.98
C16:0	22.25	34.25	0.67	0.54	0.64	0.65	27.46
C16:1n-7	8.91	-	-	-	-	-	9.07
C18:0	4.19	3.60	-	-	0.03	0.06	2.92
C18:1n-9	12.08	19.98	81.50	78.43	79.02	81.38	7.41
C18:1n-7	3.07	-	-	-	-	-	2.95
C18:2n-9	-	-	5.88	8.72	7.74	7.24	-
C18:2n-6	7.86	41.11	11.95	0.67	6.48	0.99	2.07
C18:3n-6	0.27	-	-	-	-	-	0.12
C18:3n-3	1.40	-	-	10.53	5.70	0.07	0.88
C18:4n-3	2.87	-	-	-	-	-	2.44
C20:4n-6	0.75	-	-	-	-	-	0.80
C20:5n-3	11.91	-	-	-	-	-	14.75
C22:5n-3	2.21	-	-	-	-	-	2.63
C22:6n-3	13.87	-	-	-	-	9.60	8.37
Totals							
Saturates	26.94	38.91	0.67	0.54	0.68	0.71	39.36
Monoenoics	25.78	19.98	81.50	78.43	79.02	81.38	20.37
(n-6)	8.88	41.11	11.95	0.67	6.48	0.99	2.99
(n-3)	5.58	0.00	0.00	10.53	5.70	0.07	4.49
(n-3)HUFA	28.00	0.00	0.00	0.00	0.00	9.60	25.75
Others	4.82	0.00	5.88	9.83	8.12	7.24	7.03

[1] Only saturated fatty acids, monoenoic fatty acids, fatty acids of n-9, n-6, and n-3 series are listed, so the sum of fatty acids in a column might not necessary equal 100%. A dash (-) means not detected.

[2] Means of three replicates.

[3] Conditioning diet (Zeigler Bros, Inc. Gardners, PA) used before initiation of the feeding trial.

Feed efficiency (FE = WG/TF) was calculated based on total body weight gain (WG) of fish and total dry feed (TF) offered to fish in each tank. Survival rate [$(N_i - N_d)/ N_i$] was calculated based on initial number of fish (N_i) and total number of fish that died (N_d) in each tank.

Sample Collection and Analytical Procedures

Three sample collections were made for proximate analysis and/or fatty acid analysis: (1) prior to the initiation of the feeding trial, (2) in the 4th week, and (3) at the end of the feeding trial. All analyses were done after the completion of the feeding trial.

Proximate analysis

Analysis of crude protein, lipid, ash, and moisture was performed on fish collected prior to, and at the end of, the feeding trial. Prior to the experiment, six fish were randomly selected from all available fish, and pooled. Proximate analysis was conducted in triplicate from the pooled sample of six fish. At the end of the feeding trial, one or two fish (depending on the size of the fish) were randomly selected from each tank. Fish from two randomly selected tanks that received the same treatment were pooled by block, producing two pooled samples per treatment in each block (a total of four pooled samples per treatment, two from each of the two blocks). Proximate analysis was conducted on a subsample from each of the four pooled samples from each treatment.

Fish were sacrificed and eviscerated, cut into pieces, frozen, and freeze-dried for over 24 hrs. Moisture content of fish was determined as weight loss upon drying.

The freeze-dried fish were finely ground with a food blender (Osterizer) and kept frozen (-80°C) until needed. Ash was determined by combusting freeze-dried samples (2 g) in a muffle furnace (Barnstead/Thermolyne Corp, Dubuque, Iowa) at 600°C for 2 hrs. Total lipid from 1.5 to 2-g dried-fish samples was determined by hexane extraction (Soxtec System B, Tecator AB, Hoganas, Sweden). Crude protein was determined in 0.5 g samples by the macro-Kjeldahl procedure (Kjeltec 1030, Tecator AB, Hoganes, Sweden).

Lipid analysis

Prior to initiation of the feeding trial, eight fish were randomly selected from all available fish. The selected fish were pooled in pairs and subjected to lipid analysis. At the end of the fourth week, due to the anticipated mortality of fish during the course of the study, only four fish per treatment were randomly selected (one fish from each of two randomly selected tanks per block). Lipid analysis was conducted on each of the fish sampled. At the end of the feeding trial, two or three fish from each of the eight tanks per treatment (four tanks from each block) were selected and pooled (as described in the proximate analysis section) for lipid analysis. The selected fish were sacrificed and the internal organs were removed. The fish body and liver were bagged separately and stored frozen in nitrogen at -80°C until analyzed.

Lipids were extracted from 2-g muscle samples (a strip of dorsal flesh or whole fillet if the fish was small) and 0.5-g liver samples, using chloroform:methanol (2:1 v/v with 0.02% of butylated hydroxytoluene added to the solvents as an antioxidant) by the method of Folch et al. (1957). Extracted lipids were separated into polar and neutral

fractions by thin layer chromatography (TLC). Glass plates (20 x 20 cm) coated with silica gel 150A (250 μm) were developed in petroleum ether/diethyl ether/acetic acid (85:15:0.5 v/v/v). A standard containing a mixture of polar and neutral fatty acids (Nu Chek Prep Inc., Elysian, MN) was chromatographed simultaneously. The plate with the standard was treated with iodine vapor to reveal the position of the lipid fractions. Polar and neutral lipids were recovered by scraping the silica gel from the appropriate portion of the plate and resuspending the fatty acids in chloroform:methanol (1:1 v/v). Polar and neutral lipid fractions were saponified and methylated by the method of Morrison and Smith (1964) using boron trifluoride-methanol. A known concentration of methyl tricosanoate (23:0) (Matreya Inc. Pleasant Gap, PA) was added to each sample after methylation as an internal standard for quantification of fatty acids. Fatty acids were quantified by gas chromatography/mass spectrometry based on individual peak area response of tissue fatty acids compared to the response of methyl tricosanoate. Methyl tricosanoate was chosen as the internal standard because it is not naturally present in fish oils and it does not coelute with other components in the samples. It elutes between two naturally-occurring fatty acids of interest, 20:5n-3 and 22:6n-3.

The fatty acid composition of lipids was determined using a Hewlett Packard 5890 gas chromatograph equipped with a fused-silica capillary column (30 m x 0.25 mm I.D., coated with 0.25 μm DB-23, J&W Scientific, Folsom, CA) and connected to a Hewlett Packard 5971 Mass Selective Detector (Hewlett Packard Co., Palo Alto, CA). Helium was the carrier gas at a column pressure of 7 psi with column flow rate of 0.84

ml/min. Injector temperature was 250°C. In each run, oven temperature was initially held at 180°C for 8 min, then increased to 194°C at 2°C/min and held at 194°C for 20 min. Total run time was 35 min. Sample split ratio was 80:1. Injection volume was 5 μl, which provided 5000 ng of sample on-column after the split. Fatty acid methyl esters were identified by comparison of retention times with those of known standards (Nu Chek Prep Inc., Elysian, MN) and by mass spectrometry analysis. Mass spectrometric conditions were: mass range, 50 - 550 a.m.u.; ionization voltage, 70 eV; interface temperature, 200°C; and scan rate, 4 scans/sec.

Data analysis

The experimental design was a randomized complete block. Mean weight gain, survival, feed efficiency, body protein, lipid, ash, moisture, and fatty acid concentrations of muscle and liver were compared using the General Linear Models Procedure of the Statistical Analysis Software (SAS) System for personal computers, version 6.08 (Statistical Analysis Systems 1988). Data expressed as percentages were arcsin transformed before analysis of variance (Zar 1984). Duncan's multiple range test was used to identify differences among treatment means (alpha = 0.05) when significant differences were indicated.

RESULTS

Growth Performance

The growth of fish fed the six experimental diets is presented in Table 4. In general, fish fed the MFO diet showed excellent growth response, fish fed the low fat diet showed intermediate growth response, and those fed the FAEE diets showed poor growth response.

Survival ranged from 76.2 to 93.7% (Table 4). There was no significant difference in survival rate among fish fed all diets.

Weight gain of fish ranged from 24.5 to 401.1% (Table 4). Fish fed the four FAEE diets showed no difference in weight gain. Their weight gains were lower than those of fish fed LF and MFO. Fish fed MFO had the highest weight gain. All fish appeared to grow at the same rate in the first week of the feeding trial (Figure 1). Weight gain of fish fed FAEE diets occurred most rapidly in the first two weeks and started to plateau at the fourth week. Fish fed LF also grew fastest in the first week of the feeding trial and plateaued at the eighth week. Fish fed MFO grew at about the same rate throughout the feeding trial.

Feed efficiency ratios (FE) ranged from 0.29 to 0.84 (Table 4). Feed efficiency ratio was not significantly different among fish fed the four FAEE diets. Fish fed N6, N3, and H3 had lower ($P < 0.05$) FE than fish fed LF and MFO. FE of fish fed

Table 4. Weight gain, feed efficiency ratio, and survival of red drum fingerlings fed experimental diets for nine weeks[1,2]. Anova with 5 and 36 df was used to test differences among means.

Diet	Initial Weight (g)	Weight Gain[3] %	Feed Efficiency Ratio	Survival[3] %
LF	9.85 ± 0.54	131.92 ± 11.51[b]	0.53 ± 0.04[b]	93.75 ± 4.38
N6	9.05 ± 0.75	37.43 ± 8.39[c]	0.29 ± 0.04[c]	81.25 ± 7.99
N3	9.93 ± 0.41	55.42 ± 10.54[c]	0.35 ± 0.06[c]	91.67 ± 4.45
N6+3	8.36 ± 0.90	51.98 ± 9.87[c]	0.38 ± 0.08[bc]	77.38 ± 5.45
H3	8.94 ± 0.81	24.55 ± 6.89[c]	0.30 ± 0.07[c]	76.19 ± 6.07
MFO	9.27 ± 0.51	401.10 ± 26.03[a]	0.84 ± 0.03[a]	89.58 ± 4.38
$(MSE)^{1/2}$		36.759	0.153	0.359
F value		122.95	15.19	2.35
Pr > F		0.000	0.000	0.060

[1] Means of eight replicate groups using raw data.
[2] Means with the same superscript letter in the same column are not significantly different ($P > 0.05$).
[3] $(MSE)^{1/2}$ values were calculated after arcsin transformation of data.

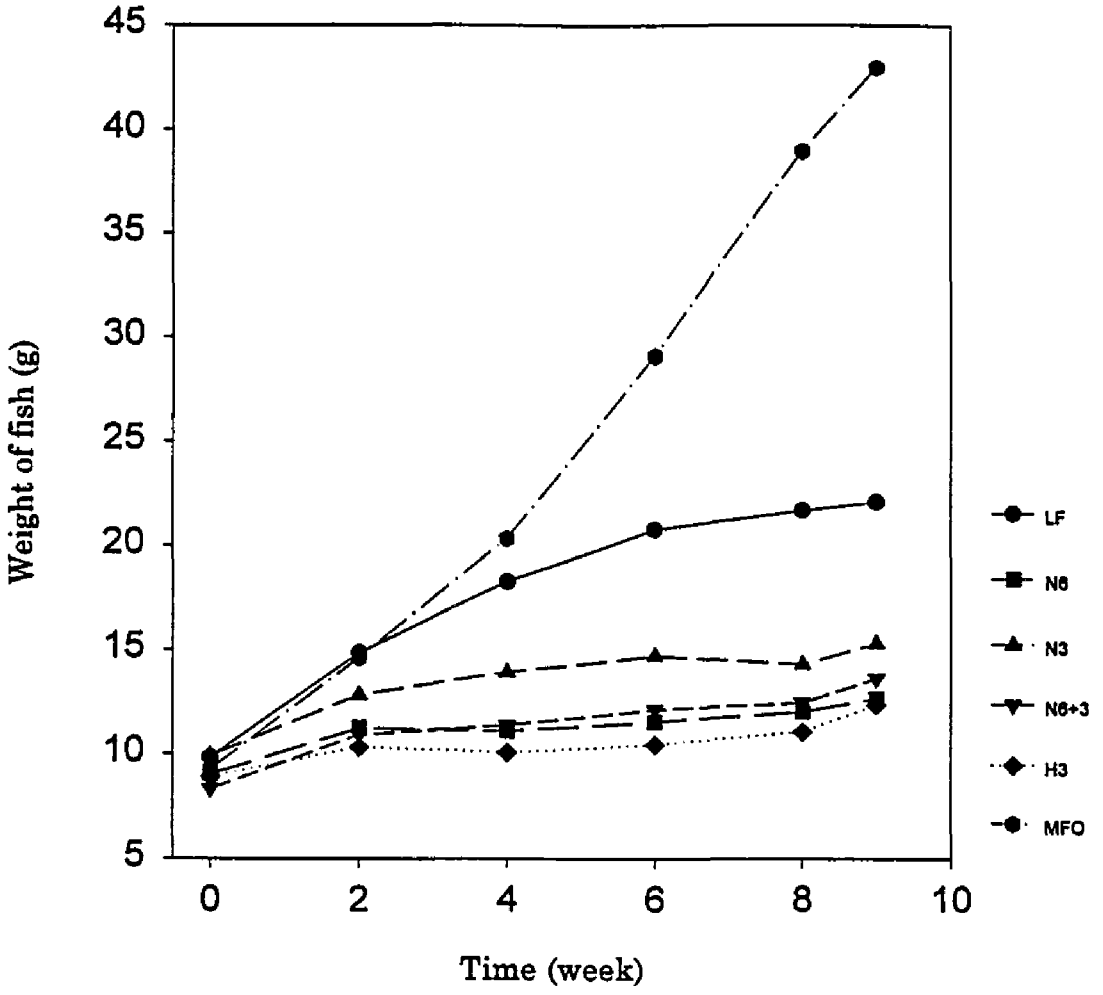


Figure 1. Average initial and biweekly weights of red drum fed six experimental diets for nine weeks. Values represent means of eight replicate groups.

N6+3 did not differ ($P > 0.05$) from FE of fish fed LF. Fish fed MFO had the highest FE.

Signs of EFA deficiency such as "shock syndrome" were not observed in this study, but fish fed FAEEs and the LF, late in the feeding trial, appeared to be rather inactive compared to fish fed MFO. Hemorrhages on the head and fins were occasionally observed in fish fed the FAEE and LF diets.

Body Composition

There was no significant difference in crude protein content of fish fed the experimental diets, regardless of dietary lipid composition (Table 5). Moisture level of fish fed the FAEE diets did not differ ($P > 0.05$). Fish fed FAEEs had significantly higher moisture content than those fed MFO or LF diets. Fish fed LF had the lowest ($P < 0.05$) moisture content. Fish fed MFO had the highest lipid level among treatments (approximately four times as much lipid as fish fed the other diets). There was no significant difference in lipid content of fish fed FAEE diets. Only in fish fed MFO was lower moisture content associated with higher ($P < 0.05$) body lipid. Ash was lower ($P < 0.05$) in fish fed MFO than in those fed N6, N3, N6+3, and H3, but not significantly different from fish fed the LF diet. Fish fed H3 had higher ($P < 0.05$) ash content than those fed LF, N3, and MFO, but did not differ ($P > 0.05$) from fish fed N6 and N6+3.

Fatty Acid Composition

The fatty acid composition of tissue lipid reflected the fatty acid composition of dietary lipid. Fatty acid (FA) composition of muscle lipids (Figures 2 and 3) showed

Table 5. Body composition of red drum fingerlings fed experimental diets for nine weeks[1,2,3] Anova with 5 and 14 (5 and 12 for moisture) df was used to test differences among means.

Diet	Moisture %	Protein[4] %	Lipid[4] %	Ash[4] %
Initial	*79.07[a]	**70.08 ± 1.03	**2.89 ± 0.08[b]	**22.17 ± 2.86[bc]
LF	62.31 ± 0.27[c]	72.55 ± 1.61	2.78 ± 0.18[b]	21.18 ± 0.25[bc]
N6	78.57 ± 0.82[a]	71.91 ± 0.83	2.56 ± 0.15[b]	25.00 ± 1.19[ab]
N3	78.53 ± 0.17[a]	71.32 ± 1.28	2.73 ± 0.15[b]	22.76 ± 0.83[b]
N6+3	79.00 ± 0.43[a]	71.21 ± 2.12	2.86 ± 0.57[b]	26.94 ± 2.51[ab]
H3	79.61 ± 0.46[a]	70.10 ± 1.06	2.56 ± 0.18[b]	28.82 ± 2.36[a]
MFO	74.69 ± 0.19[b]	68.83 ± 0.78	10.85 ± 0.32[a]	16.65 ± 0.36[c]
$(MSE)^{1/2}$	0.015	0.338	0.0052	0.038
F value	129.54	1.27	166.98	5.53
Pr > F	0.000	0.329	0.000	0.004

[1] Means of four replicates, *one replicate, ** three replicates, using raw data.
[2] Means with the same superscript letter in the same column are not significantly different (P > 0.05)
[3] $(MSE)^{1/2}$ values were calculated after arcsin transformation of data.
[4] Dry weight basis.

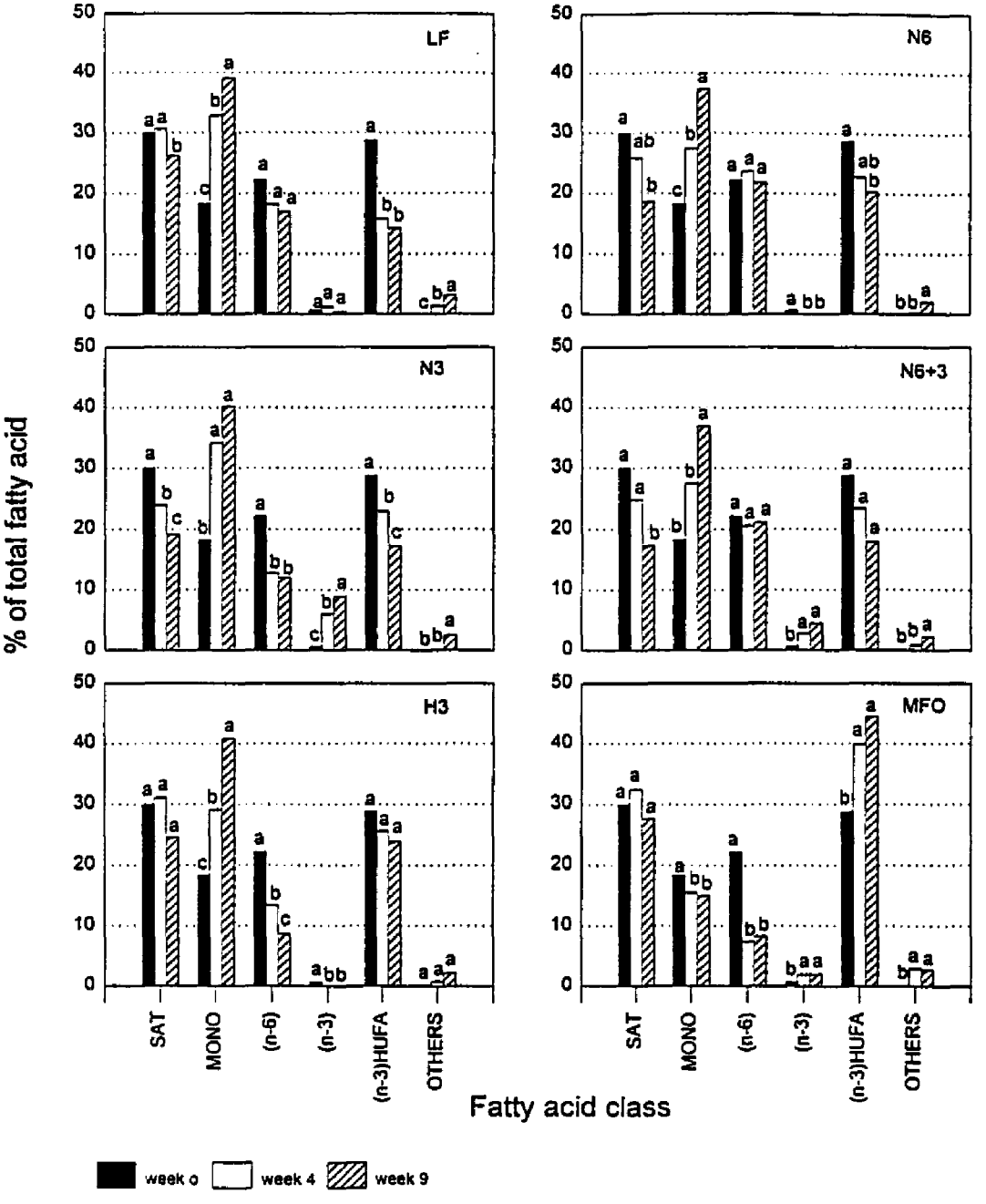


Figure 2. Comparison of fatty acid profile in muscle polar lipid of red drum fed six experimental diets for zero, four, and nine weeks. Means with the same letter in the same fatty acid class in the same diet are not significantly different ($P > 0.05$).

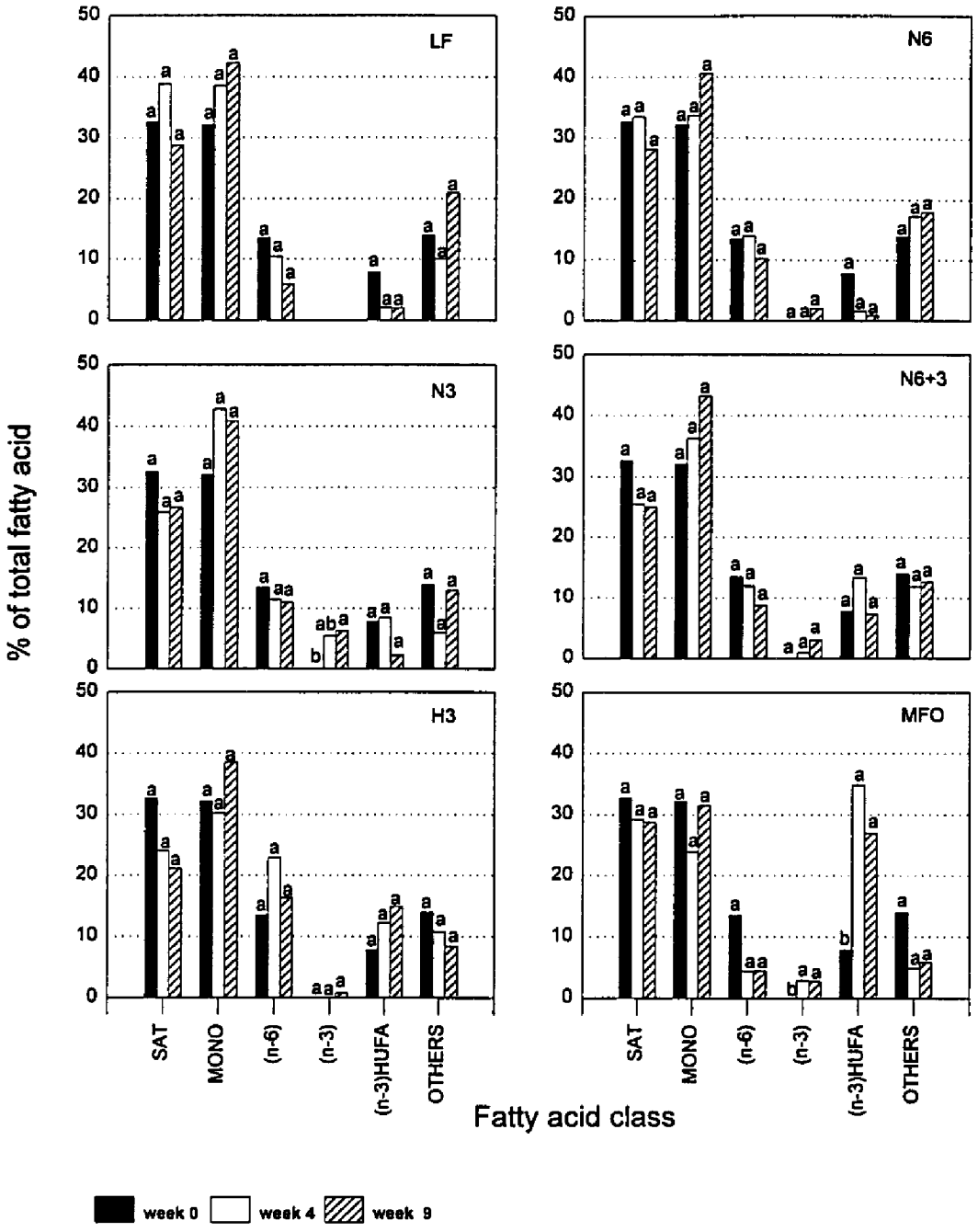


Figure 3. Comparison of fatty acid profile in muscle nonpolar lipid of red drum fed six experimental diets for zero, four, and nine weeks. Means with the same letter in the same fatty acid class in the same diet are not significantly different ($P > 0.05$).

trends similar to those in liver lipids (Figures 4 and 5). Although quantification of polar and neutral lipids was not performed, it was observed from TLC plates and GC chromatographs that the polar portion of muscle lipid was greater than the nonpolar portion, while in liver, the nonpolar portion was greater. The level of n-3 highly unsaturated fatty acids was higher in polar lipid than in nonpolar lipid in both muscle and liver tissues.

Saturated fatty acids (SFAs)

Muscle polar lipid: Total concentrations of SFAs in muscle polar lipid in fish fed LF, N6, N3 and N6+3 decreased significantly from week-0 to week-9, while fish fed H3 and MFO had no significant change in total SFAs during the nine week feeding period (Figure 2). Total SFAs ranged from 23.9 to 32.4% in week four (Table 6). Fish fed LF, H3, and MFO had higher SFAs levels ($P < 0.05$) than fish fed N6, N3, N6+3 (Table 6). In week nine, total SFAs ranged from 17.3 to 27.6% (Table 7). There was no significant difference in total SFAs among fish fed all diets (Table 7).

Muscle nonpolar lipid: Levels of total SFAs in muscle nonpolar lipid were stable over the 9-week feeding trial. There was no significant change in SFA concentration throughout the feeding period in fish fed the six experimental diets (Figure 3). In week four, total SFAs ranged from 24.0 to 38.8% (Table 8) and in week nine, from 21.1 to 28.7% (Table 9). SFA concentrations in muscle nonpolar lipid were not significantly different among treatments in week four or week nine (Tables 8, 9).

Liver polar lipid: Levels of total SFAs in liver polar lipid were similar to those in muscle polar lipid. There was a decrease ($P < 0.05$) in the level of total SFAs

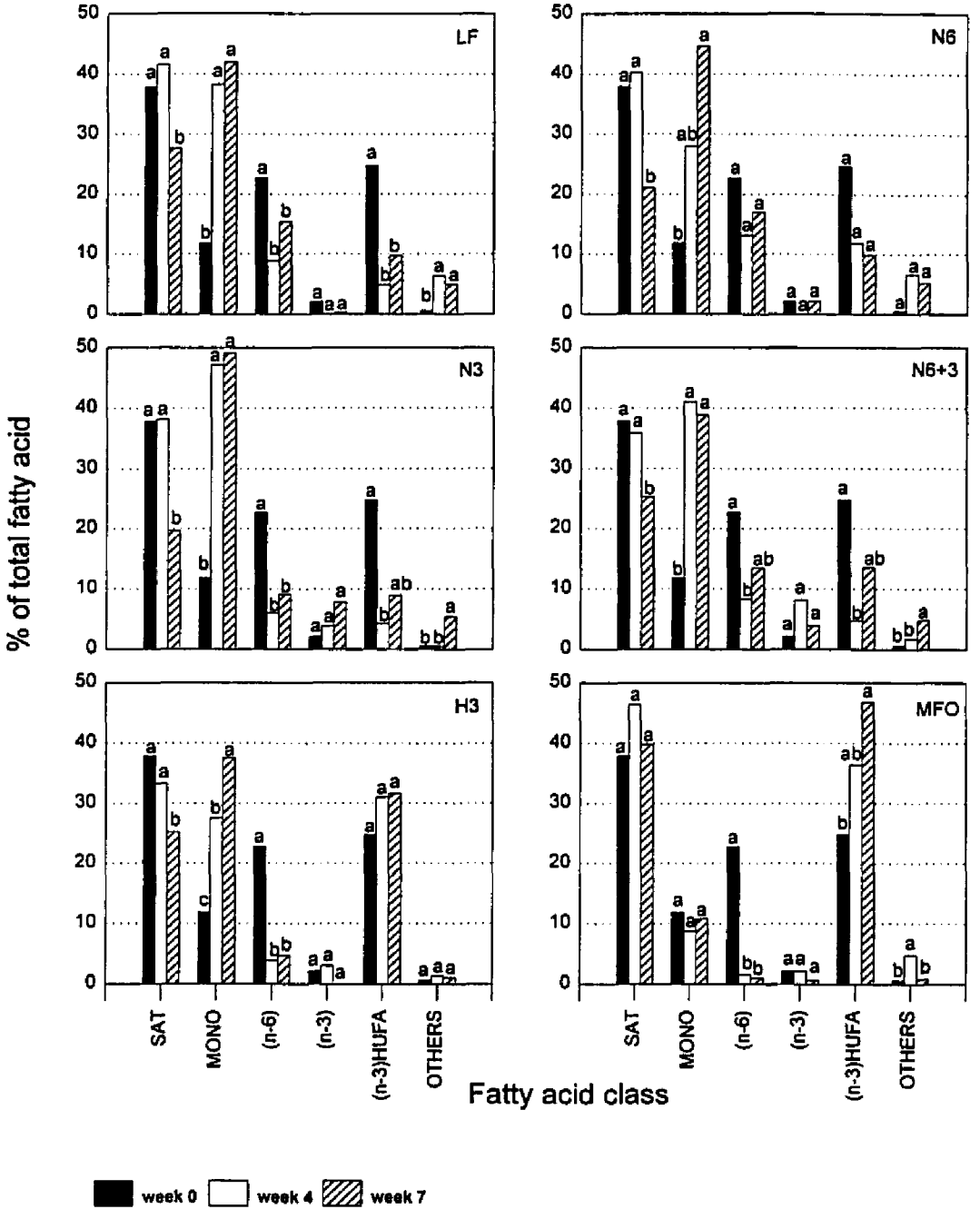


Figure 4. Comparison of fatty acid profile in liver polar lipid of red drum fed six experimental diets for zero, four, and nine weeks. Means with the same letter in the same fatty acid class in the same diet are not significantly different ($P > 0.05$).

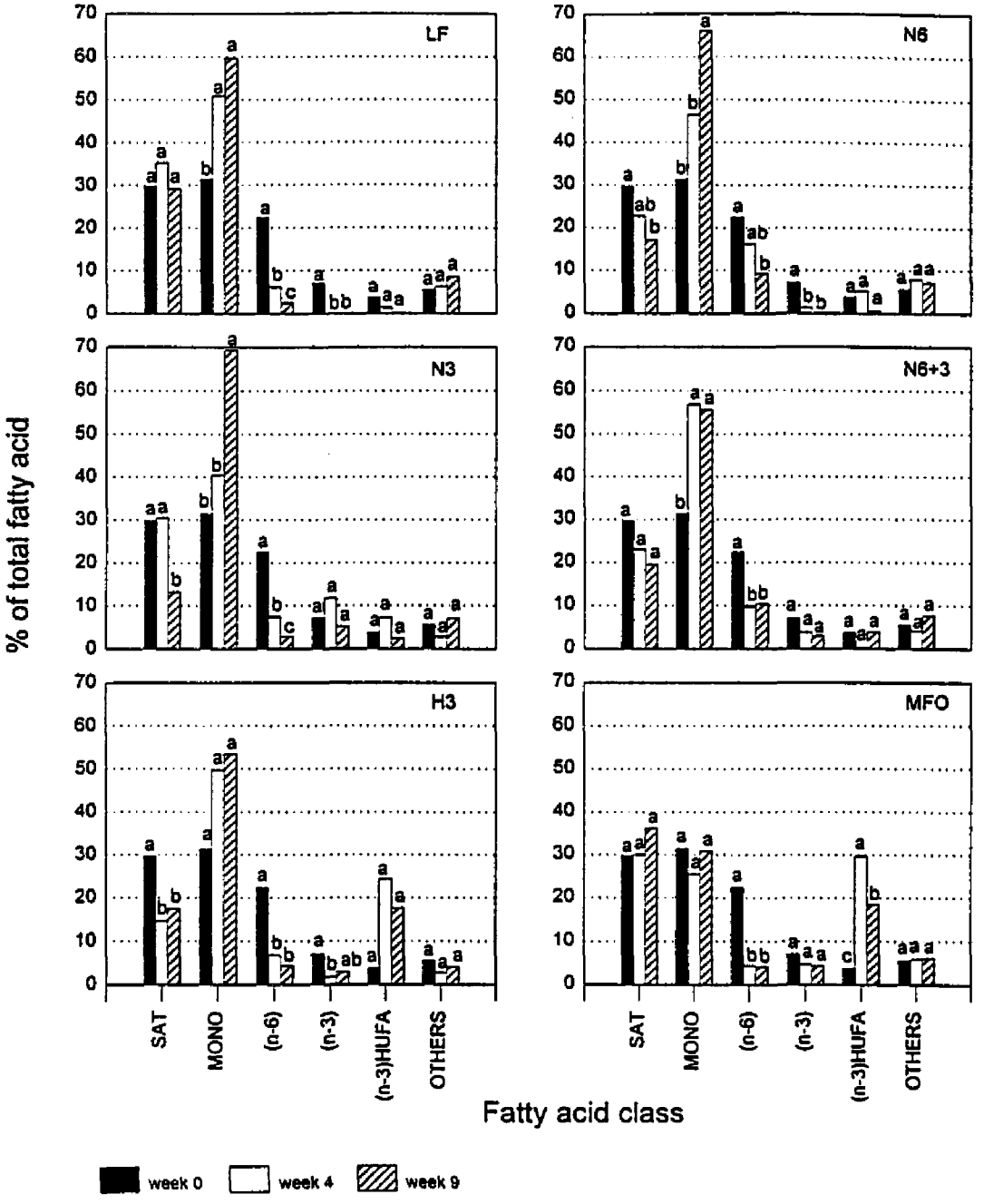


Figure 5. Comparison of fatty acid profile in liver nonpolar lipid of red drum fed six experimental diets for zero, four, and nine weeks. Means with the same letter in the same fatty acid class in the same diet are not significantly different ($P > 0.05$).

Table 6. Fatty acid composition (% of total fatty acids) of muscle polar lipid in red drum fingerlings fed experimental diets for four weeks[1,2,3]. Anova with 5 and 12 df was used to test differences among means.

Fatty acid	Initial	Diet							F	
		LF	N6	N3	N6+3	H3	MFO	(MSE)[4]	value	Pr > F
C14:0	0.52	0.68 ± 0.23[b]	0.18 ± 0.20[b]	0.35 ± 0.13[b]	0.61 ± 0.10[b]	0.50 ± 0.19[b]	1.46 ± 0.18[a]	0.003	7.03	0.003
C16:0	22.16	23.08 ± 0.91[ab]	19.62 ± 0.88[bc]	17.75 ± 0.30[c]	19.08 ± 1.89[bc]	23.73 ± 1.08[a]	24.24 ± 2.33[a]	0.026	4.85	0.012
C16:1n-7	1.81	8.11 ± 0.76[a]	1.17 ± 0.67[c]	1.71 ± 0.07[c]	1.89 ± 0.20[bc]	1.52 ± 0.54[c]	3.10 ± 0.18[b]	0.008	44.62	0.000
C18:0	7.29	6.89 ± 0.46[a]	6.02 ± 0.25[ab]	5.81 ± 0.36[ab]	5.15 ± 0.40[b]	6.84 ± 0.40[a]	6.72 ± 0.55[a]	0.007	3.61	0.032
C18:1n-9	14.52	20.44 ± 1.85[b]	25.06 ± 1.85[ab]	29.90 ± 2.60[a]	24.10 ± 3.81[ab]	25.46 ± 2.04[ab]	9.50 ± 0.44[c]	0.049	8.55	0.001
C18:1n-7	1.69	2.44 ± 0.17[a]	0.62 ± 0.38[c]	1.39 ± 0.11[b]	0.79 ± 0.27[bc]	1.50 ± 0.12[b]	2.64 ± 0.17[a]	0.005	12.47	0.000
C18:2n-9	-	1.35 ± 0.30[a]	0.13 ± 0.13[b]	-	0.35 ± 0.20[b]	-	-	0.003	15.39	0.000
C18:2n-6	19.24	14.61 ± 1.55[bc]	20.28 ± 1.32[a]	10.93 ± 1.40[c]	16.79 ± 1.91[ab]	11.72 ± 0.50[c]	5.32 ± 0.53[d]	0.025	16.90	0.000
C18:3n-6	-	0.43 ± 0.26[a]	0.12 ± 0.12[b]	-	0.23 ± 0.15[ab]	-	-	0.002	3.69	0.030
C18:3n-3	0.68	0.75 ± 0.06[a]	-	6.01 ± 0.59[a]	2.69 ± 0.49[b]	-	0.43 ± 0.16[c]	0.007	44.44	0.000
C18:4n-3	-	0.43 ± 0.43	-	-	0.11 ± 0.11	-	0.66 + 0.23	0.004	1.83	0.181
C20:1n-9	0.29	1.90 ± 1.23	0.59 ± 0.20	1.18 ± 0.10	0.71 ± 0.20	0.64 ± 0.22	0.23 ± 0.13	0.011	1.20	0.365
C20:2n-6	-	-	0.15 ± 0.15	-	-	-	-	0.001	1.00	0.458
C20:3n-6	-	-	-		0.07 ± 0.07	-	0.12 ± 0.12	0.001	0.83	0.554
C20:4n-6	2.93	3.15 ± 1.65	3.09 ± 0.40	1.84 ± 0.26	3.49 ± 1.20	1.71 ± 0.60	1.93 ± 0.07	0.018	0.76	0.596
C20:4n-3	-	-	-	-	-	-	0.83 ± 0.03	0.000	950.16	0.000
C20:5n-3	6.97	3.47 ± 1.36[c]	6.01 ± 0.22[bc]	5.13 ± 0.44[bc]	7.45 ± 1.06[b]	5.10 ± 0.53[bc]	13.10 ± 0.81[a]	0.019	13.52	0.000
C22:5n-3	2.50	2.08 ± 0.85[b]	1.14 ± 0.47[b]	1.92 ± 0.24[b]	1.79 ± 0.32[b]	0.63 ± 0.38[b]	4.55 ± 0.66[a]	0.012	5.57	0.007
C22:6n-3	19.30	10.18 ± 2.14[c]	15.69 ± 1.96[bc]	15.94 ± 1.08[bc]	14.20 ± 2.06[bc]	19.86 ± 1.21[ab]	22.24 ± 3.09[a]	0.034	7.38	0.003
Totals										
Saturates	29.97	30.66 ± 1.36[a]	25.85 ± 1.06[b]	23.91 ± 0.47[b]	24.85 ± 1.98[b]	31.07 ± 1.08[a]	32.42 ± 2.98[a]	0.027	7.93	0.002
Monoenoics	18.31	32.89 ± 2.32[a]	27.43 ± 1.04[a]	34.18 ± 2.84[a]	27.50 ± 3.85[a]	29.11 ± 2.06[a]	15.47 ± 0.62[b]	0.054	6.58	0.004
(n-6)	22.17	18.19 ± 2.04[ab]	23.65 ± 1.77[a]	12.77 ± 1.65[b]	20.58 ± 2.34[a]	13.43 ± 0.90[b]	7.37 ± 0.53[a]	0.035	11.95	0.000
(n-3)	0.68	1.19 ± 0.46[c]	-	6.01 ± 0.59[a]	2.80 ± 0.58[b]	-	1.92 ± 0.11[bc]	0.008	28.64	0.000
(n-3)HUFA[4]	28.77	15.73 ± 1.94[c]	22.84 ± 2.19[bc]	22.99 ± 1.50[bc]	23.43 ± 3.13[bc]	25.60 ± 1.66[b]	39.89 ± 4.51[a]	0.052	10.46	0.001
Others[5]	0.10	1.35 ± 0.30[b]	0.23 ± 0.23[b]	0.15 ± 0.15[b]	0.85 ± 0.39[b]	0.80 ± 0.52[b]	2.92 ± 0.46[a]	0.008	7.07	0.003

[1] Means of four samples using raw data. (MSE)[4] values were calculated after arcsin transformation of data.

[2] Means with the same letters in the same row are not significantly different (P>0.05).

[3] A dash (-) means not detected.

[4] Highly unsaturated fatty acids of the (n-3) series with five or more double bonds.

[5] Include 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-4, 18:3n-4, 18:4n-1.

Table 7. Fatty acid composition (% of total fatty acids) of muscle polar lipid in red drum fingerlings fed experimental diets for nine weeks[1,2,3]. Anova with 5 and 12 df was used to test differences among means.

Fatty acid	Initial	Diet						(MSE)$^{1/2}$	F value	Pr > F
		LF	N6	N3	N6+3	H3	MFO			
C14:0	0.52	0.48 ± 0.32^{abc}	0.18 ± 0.18^{c}	0.32 ± 0.19^{bc}	0.14 ± 0.14^{c}	0.90 ± 0.30^{ab}	1.05 ± 0.15^{a}	0.004	3.39	0.039
C16:0	22.16	19.41 ± 0.52	13.49 ± 4.09	14.18 ± 0.59	12.46 ± 3.35	16.97 ± 2.16	20.11 ± 1.42	0.050	1.70	0.208
C16:1n-7	1.81	11.87 ± 1.53^{a}	2.66 ± 1.04^{b}	2.97 ± 0.38^{b}	2.08 ± 0.95^{b}	4.11 ± 2.14^{b}	3.38 ± 0.47^{b}	0.026	8.28	0.001
C18:0	7.29	6.31 ± 0.82	4.91 ± 0.51	4.65 ± 0.43	4.68 ± 0.41	6.63 ± 1.94	6.27 ± 0.40	0.018	1.01	0.455
C18:1n-9	14.52	24.34 ± 1.45^{b}	33.42 ± 2.35^{a}	34.66 ± 1.27^{a}	32.44 ± 1.45^{a}	35.33 ± 0.85^{a}	8.72 ± 0.25^{c}	0.032	44.86	0.000
C18:1n-7	1.69	2.68 ± 0.09^{a}	0.55 ± 0.33^{bc}	1.44 ± 0.78^{b}	0.96 ± 0.35^{bc}	0.15 ± 0.15^{c}	2.63 ± 0.06^{a}	0.007	10.33	0.001
C18:2n-9	-	3.15 ± 0.15	1.86 ± 0.26	2.00 ± 0.74	1.25 ± 0.73	0.20 ± 0.12	-	0.020	0.99	0.464
C18:2n-6	19.24	14.78 ± 0.28^{ab}	16.95 ± 5.68^{a}	9.19 ± 0.82^{bc}	17.65 ± 1.48^{a}	7.21 ± 0.88^{c}	2.89 + 0.05^{c}	0.047	6.50	0.004
C18:3n-6	-	1.11 ± 0.48^{a}	1.03 ± 0.35^{ab}	-	0.17 ± 0.17^{bc}	-	0.34 ± 0.34^{abc}	0.005	3.55	0.034
C18:3n-3	0.68	0.38 ± 0.23^{c}	-	8.68 ± 0.53^{a}	4.45 ± 0.51^{b}	-	0.52 ± 0.20^{c}	0.006	131.39	0.000
C18:4n-3	-	-	-	0.22 ± 0.13	-	-	0.30 ± 0.18	0.000	2.37	0.102
C20:1n-9	0.29	0.17 ± 0.17	0.65 ± 0.37	1.10 ± 0.30	1.43 ± 0.50	1.25 ± 0.44	0.27 ± 0.18	0.007	2.32	0.107
C20:2n-6	-	0.19 ± 0.19	0.33 ± 0.19	-	0.38 ± 0.26	-	2.43 ± 2.43	0.020	0.86	0.536
C20:3n-6	-	0.11 ± 0.11	-	-	0.09 ± 0.09	-	0.22 ± 0.14	0.001	1.79	0.190
C20:4n-6	2.93	0.78 ± 0.46^{c}	3.48 ± 0.17^{a}	2.78 ± 0.36^{a}	2.87 ± 0.09^{a}	1.45 ± 0.49^{bc}	2.41 ± 0.05^{ab}	0.007	7.60	0.002
C20:4n-3	-	-	-	-	-	-	1.17 ± 0.19^{a}	0.002	29.98	0.000
C20:5n-3	6.97	5.46 ± 0.84^{b}	4.93 ± 0.21^{b}	4.27 ± 0.24^{bc}	5.17 ± 0.45^{b}	1.88 ± 0.73^{c}	15.94 ± 1.58^{a}	0.018	28.55	0.000
C22:5n-3	2.50	0.34 ± 0.34^{b}	0.73 ± 0.44^{b}	1.22 ± 0.24^{b}	0.92 ± 0.55^{b}	0.22 ± 0.22^{b}	5.71 ± 0.19^{a}	0.008	25.67	0.000
C22:6n-3	19.30	8.45 ± 0.93^{c}	14.72 ± 1.61^{bc}	11.81 ± 0.47^{c}	11.93 ± 1.79^{c}	21.75 ± 4.94^{ab}	22.82 ± 0.39^{a}	0.048	6.07	0.005
Totals										
Saturates	29.97	26.20 ± 0.49	18.58 ± 3.68	19.15 ± 0.56	17.28 ± 3.04	24.50 ± 4.36	27.59 ± 1.38	0.055	2.71	0.073
Monoenoics	18.31	39.06 ± 1.36^{a}	37.27 ± 2.74^{a}	40.17 ± 0.90^{a}	36.92 ± 1.21^{a}	40.82 ± 1.77^{a}	14.99 ± 0.38^{b}	0.040	25.83	0.000
(n-6)	22.17	16.97 ± 1.02^{ab}	21.78 ± 5.45^{a}	11.97 ± 0.65^{bc}	21.14 ± 1.33^{a}	8.67 ± 1.32^{c}	8.27 ± 2.47^{c}	0.048	6.45	0.004
(n-3)	0.68	0.38 ± 0.23^{d}	-	8.90 ± 0.66^{a}	4.45 ± 0.51^{b}	-	1.99 ± 0.10^{c}	0.006	164.12	0.000
(n-3)HUFA[4]	28.77	14.24 ± 1.28^{b}	20.38 ± 1.99^{b}	17.29 ± 0.44^{b}	18.00 ± 2.65^{b}	23.85 ± 5.69^{b}	44.48 ± 1.44^{a}	0.062	13.92	0.000
Others[5]	0.10	3.15 ± 0.15	1.99 ± 0.22	2.52 ± 0.57	2.22 ± 0.43^{a}	2.16 ± 1.31	2.67 ± 0.58	0.014	0.37	0.858

[1] Means of four samples using raw data. (MSE)$^{1/2}$ values were calculated after arcsin transformation of data.

[2] Means with the same letters in the same row are not significantly different (P > 0.05).

[3] A dash (-) means not detected.

[4] Highly unsaturated fatty acids of the (n-3) series with five or more double bonds.

[5] Include 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-4, 18:3n-4, 18:4n-1.

Table 8. Fatty acid composition (% of total fatty acid) of muscle nonpolar lipid in red drum fingerlings fed experimental diets for four weeks[1,2,3]. Anova with 5 and 12 df was used to test differences among means.

Fatty acid	Initial	Diet						F (MSE)[½]	F value	Pr > F
		LF	N6	N3	N6+3	H3	MFO			
C14:0	-	0.51 ± 0.51	0.38 ± 0.38	0.46 ± 0.31	3.13 ±3.13	0.74 ± 0.74	2.69 ± 1.55	0.031	0.64	0.671
C16:0	22.52	28.79 ± 4.46	26.84 ± 4.98	18.57 ± 2.80	13.59 ± 4.94	17.94 ± 2.88	20.96 ± 1.06	0.077	2.37	0.102
C16:1n-7	5.20	11.25 ± 1.82[a]	1.12 ± 1.12[b]	4.20 ± 1.47[b]	0.77 ± 0.77[b]	0.95 ± 0.95[b]	9.51 ± 0.81	0.025	13.73	0.000
C18:0	10.14	9.57 ± 1.86	6.21 ± 3.32	6.87 ± 3.17	8.80 ± 1.98	5.34 ± 1.79	5.54 ± 0.31[a]	0.047	0.56	0.732
C18:1n-9	23.39	23.97 ± 2.79[ab]	31.20 ± 2.24[a]	35.83 ± 3.48[a]	35.07 9.02[a]	26.48 ± 6.87[ab]	9.81 ± 0.29[b]	0.110	3.33	0.041
C18:1n-7	2.74	3.39 ± 0.22[a]	0.69 ± 0.69[b]	1.23 ± 0.72[b]	-	-	3.51 ± 0.17[a]	0.009	12.18	0.000
C18:2n-9	13.96	9.60 ± 4.20	14.93 ± 9.14	4.55 ± 2.91	11.90 ± 6.21	10.71 ± 5.77	1.07 ± 0.63	0.130	0.63	0.682
C18:2n-6	12.78	8.89 ± 0.91[b]	13.94 ± 0.97[a]	9.67 ± 1.49[ab]	11.54 ± 1.75[ab]	7.20 ± 2.43[bc]	3.29 ± 0.08[c]	0.029	6.59	0.004
C18:3n-6	0.71	1.54 ± 0.93	-	0.98 ± 0.98	0.44 ± 0.44	-	-	0.013	1.04	0.439
C18:3n-3	-	-	-	5.43 ± 0.99[a]	0.89 ± 0.89[b]	-	-	0.009	22.19	0.000
C18:4n-3	-	-	-	-	-	-	1.37 ± 0.46	0.004	7.38	0.002
C20:1n-9	0.77	-	0.67 ± 0.67	1.52 ± 0.89	0.42 ± 0.42	2.76 ± 1.94	1.04 ± 0.41	0.016	1.49	0.264
C20:2n-6	-	-	-	-	-	-	-	-	-	-
C20:3n-6	-	-	-	-	-	2.78 ± 2.78	-	0.023	1.00	0.458
C20:4n-6	-	-	-	0.77 ± 0.77[b]	-	12.86 ± 9.23[a]	1.08 ± 0.39[b]	0.058	3.22	0.045
C20:4n-3	-	-	-	-	-	-	1.50 ± 0.50[a]	0.004	9.44	0.001
C20:5n-3	2.52	0.49 ± 0.49[b]	0.43 ± 0.43[b]	2.68 ± 1.29[b]	4.29 ± 3.23[b]	2.55 ± 2.55[b]	14.78 ± 0.61[a]	0.037	8.72	0.001
C22:5n-3	-	-	-	-	-	-	7.13 ± 0.44[a]	0.004	186.95	0.000
C22:6n-3	5.27	1.56 ± 1.56	1.17 ± 1.17	5.76 ± 1.29	9.16 ± 3.60	9.68 ± 5.61	12.91 ± 1.17	0.067	1.98	0.155
Totals										
Saturates	32.66	38.87 ± 6.14	33.43 ± 8.03	25.90 ± 5.83	25.51 ± 4.30	24.02 ± 4.77	29.19 ± 0.50	0.014	1.05	0.433
Monoenoics	32.09	38.60 ± 3.40	33.67 ± 4.29	42.78 ± 6.08	36.26 ± 10.10	30.20 ± 5.41	23.87 ± 0.73	0.133	1.17	0.378
(n-6)	13.49	10.43 ± 1.71[b]	13.94 ± 0.97[ab]	11.42 ± 0.79[b]	11.98 ± 1.64[b]	22.84 ± 9.59[a]	4.37 ± 0.37[b]	0.069	3.24	0.044
(n-3)	-	-	-	5.43 ± 0.99[a]	0.89 ± 0.89[c]	-	2.87 ± 0.96[b]	0.012	12.97	0.000
(n-3)HUFA[4]	7.79	2.06 ± 2.06[b]	1.60 ± 1.60[b]	8.44 ± 1.41[b]	13.46 ± 5.17[a]	12.23 ± 7.24[b]	34.81 ± 2.06[a]	0.093	7.11	0.003
Others[5]	13.96	10.04 ± 3.88	17.35 ± 8.08	6.04 ± 2.48	11.90 ± 6.21	10.71 ± 5.77	4.88 ± 1.10	0.125	0.54	0.746

[1] Means of four samples using raw data. (MSE)[½] values were calculated after arcsin transformation of data.

[2] Means with the same letters in the same row are not significantly different (P > 0.05).

[3] A dash (-) means not detected.

[4] Highly unsaturated fatty acids of the (n-3) series with five or more double bonds.

[5] Include 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-4, 18:3n-4, 18:4n-1.

Table 9. Fatty acid composition (% of total fatty acids) of muscle nonpolar lipid in red drum fingerlings fed experimental diets for nine weeks[1,2,3]. Anova with 5 and 12 df was used to test differences among means.

Fatty acid	Initial	Diet						(MSE)[6]	F value	Pr > F
		LF	N6	N3	N6+3	H3	MFO			
C14:0	-	0.89 ± 0.89	1.42 ± 1.42	1.94 ± 0.69	1.28 ± 1.28	1.62 ± 0.70	4.17 ± 0.78	0.021	1.26	0.341
C16:0	22.52	22.29 ± 2.62	18.18 ± 1.05	13.92 ± 2.84	17.18 ± 3.10	13.87 ± 1.42	19.90 ± 2.90	0.050	1.85	0.177
C16:1n-7	5.20	12.07 ± 4.25[a]	2.62 ± 1.54[b]	5.09 ± 0.53[b]	4.37 ± 2.00[b]	3.42 ± 0.30[b]	8.51 ± 1.02[ab]	0.042	2.90	0.061
C18:0	10.14	5.62 ± 1.99	8.57 ± 1.47	7.44 ± 2.78	6.49 ± 1.61	5.65 ± 0.57	4.67 ± 0.78	0.037	0.59	0.706
C18:1n-9	23.39	23.71 ± 1.71[ab]	37.27 ± 3.75[a]	28.60 ± 10.56[a]	30.91 ± 6.24[a]	32.74 ± 3.96[a]	10.26 ± 1.19[b]	0.110	3.19	0.046
C18:1n-7	2.74	2.32 ± 1.41[ab]	-	-	-	0.27 ± 0.27[b]	3.01 ± 0.31[a]	0.014	3.69	0.030
C18:2n-9	13.96	18.09 ± 10.92	12.48 ± 2.92	9.63 ± 5.59	11.29 ± 1.84	5.52 ± 3.48	1.38 ± 1.05	0.122	0.95	0.485
C18:2n-6	12.78	5.85 ± 2.02[ab]	7.05 ± 2.78[a]	4.30 ± 0.18[ab]	7.28 ± 0.81[a]	7.23 ± 0.90[a]	2.67 ± 0.26[b]	0.021	3.33	0.041
C18:3n-6	0.71	-	-	0.63 ± 0.63	-	-	-	0.005	1.00	0.458
C18:3n-3	-	-	-	5.50 ± 3.19[a]	1.64 ± 1.07[b]	-	0.60 ± 0.35[b]	0.023	3.57	0.033
C18:4n-3	-	-	0.74 ± 0.74	-	-	-	1.27 ± 0.48	0.007	2.45	0.094
C20:1n-9	0.77	4.23 ± 4.23	0.79 ± 0.79	6.20 ± 2.49	7.92 ± 6.80	2.11 ± 0.78	9.68 ± 8.89	0.103	0.46	0.799
C20:2n-6	-	-	-	-		-	0.93 ± 0.93	0.008	1.00	0.458
C20:3n-6	-	-	3.16 ± 3.16	0.67 ± 0.67	-	0.04 ± 0.04	-	0.026	0.91	0.505
C20:4n-6	-	-	-	5.39 ± 2.47	1.47 ± 1.47	9.09 ± 7.72	0.83 ± 0.38	0.068	1.19	0.370
C20:4n-3	-	-	1.31 ± 1.31	0.80 ± 0.80	1.37 ± 1.37	0.73 ± 0.73	0.79 ± 0.52	0.018	0.30	0.902
C20:5n-3	2.52	0.58 ± 0.58[b]	0.89 ± 0.89[b]	1.09 ± 1.09[b]	1.57 ± 1.57[b]	1.42 ± 0.84[b]	11.85 ± 1.50[a]	0.022	16.26	0.000
C22:5n-3	-	0.34 ± 0.34	-	-	5.79 ± 5.79	0.20 ± 0.20	6.20 ± 1.06	0.048	1.60	0.233
C22:6n-3	5.27	1.10 ± 1.10[b]	-	1.15 ± 0.69[b]	-	13.27 ± 4.60[a]	8.87 ± 1.33[a]	0.032	12.63	0.000
Totals										
Saturates	32.66	28.81 ± 4.76	28.16 ± 2.51	26.71 ± 4.96	24.95 ± 5.20	21.14 ± 1.26	28.74 ± 4.31	0.080	0.59	0.706
Monoenoics	32.09	42.34 ± 3.31	40.69 ± 4.26	40.84 ± 10.51	43.21 ± 2.98	38.54 ± 3.40	31.46 ± 6.41	0.134	0.46	0.795
(n-6)	13.49	5.85 ± 2.02	10.21 ± 3.79	10.99 ± 1.67	8.76 ± 2.12[a]	16.37 ± 8.29	4.44 ± 1.02	0.088	0.98	0.470
(n-3)	-	-	2.05 ± 2.05	6.30 ± 3.03	3.02 ± 2.38	0.73 ± 0.73	2.66 ± 0.82	0.036	1.53	0.252
(n-3)HUFA[4]	7.79	2.02 ± 2.02[c]	0.89 ± 0.89[c]	2.24 ± 1.68[c]	7.36 ± 5.47[bc]	14.89 ± 5.39[b]	26.92 ± 2.44[a]	0.056	13.61	0.000
Others[5]	13.96	20.99 ± 10.11	18.00 ± 6.06	12.91 ± 7.15	12.72 ± 2.25	8.33 ± 4.35	5.78 ± 0.75	0.139	0.72	0.621

[1] Means of four samples using raw data. (MSE)[6] values were calculated after arcsin transformation of data.

[2] Means with the same letters in the same row are not significantly different (P > 0.05).

[3] A dash (-) means not detected.

[4] Highly unsaturated fatty acids of the (n-3) series with five or more double bonds.

[5] Include 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-4, 18:3n-4, 18:4n-1.

during the 9-week growth trial in all fish except those fed MFO (Figure 4). In week four, total SFAs ranged from 33.3 to 46.4% (Table 10). There was no significant difference in the level of total SFAs in fish fed the six diets (Table 10). In week nine, total SFAs in fish ranged from 19.7 to 39.8% (Table 11). Fish fed MFO were significantly higher ($P < 0.05$) in SFAs than those fed LF and FAEEs (N6, N3, N6+3 and H3) (Table 11). Fish fed N6 and N3 had lower ($P < 0.05$) levels of SFAs in liver polar lipid than those fed LF, but did not differ ($P > 0.05$) from those fed N6+3 and H3.

Liver nonpolar lipid: In liver nonpolar lipid, there was a decrease ($P < 0.05$) of total SFAs in fish fed N6, N3 and H3 during the 9-week period, but not in fish fed LF, N6+3 and MFO (Figure 5). In week four, total SFAs ranged from 14.7 to 35.3% (Table 12). SFAs were highest ($P < 0.05$) in fish fed LF, and lowest ($P < 0.05$) in fish fed H3 (Table 12). Fish fed N6, N3, N6+3, and MFO did not differ ($P > 0.05$). In week nine, total SFAs ranged from 13.2 to 36.1% (Table 13). SFAs were higher ($P < 0.05$) in fish fed LF and MFO than in those fed FAEEs. There was no significant difference in SFA content of nonpolar liver lipid among fish fed FAEEs (Table 13).

For all fish, SFAs were composed primarily of palmitic acid (16:0), followed by oleic acid (18:0) and myristic acid (14:0). Other saturated fatty acids were not detected.

Table 10. Fatty acid composition (% of total fatty acids) of liver polar lipid in red drum fingerlings fed experimental diets for four weeks[1,2,3]. Anova with 5 and 10 df was used to test differences among means.

Fatty acid	Initial	Diet						(MSE)$^{1/2}$	F value	Pr > F
		LF	N6	N3*	N6+3*	H3	MFO			
C14:0	1.08	1.36 ± 0.77^{b}	-	0.13 ± 0.13^{b}	0.94 ± 0.94^{b}	-	4.98 ± 1.32^{a}	0.010	13.18	0.000
C16:0	30.71	30.44 ± 0.72	27.39 ± 5.73	27.14 ± 6.45	25.70 ± 4.58	27.65 ± 2.52	30.90 ± 1.64	0.069	0.51	0.764
C16:1n-7	2.23	12.29 ± 4.54^{a}	-	1.08 ± 1.08^{b}	1.26 ± 1.26^{b}	-	5.81 ± 0.80^{ab}	0.047	4.25	0.025
C18:0	6.09	9.88 ± 1.33	12.83 ± 2.06	10.98 ± 2.41	9.26 ± 1.49	5.63 ± 2.05	10.50 ± 1.24	0.037	1.69	0.225
C18:1n-9	8.53	22.98 ± 3.47^{c}	24.68 ± 9.46^{bc}	45.30 ± 5.28^{a}	39.78 ± 4.82^{ab}	25.28 ± 1.88^{bc}	2.91 ± 1.00^{d}	0.098	9.42	0.002
C18:1n-7	1.09	2.65 ± 0.96^{a}	-	-	-	-	-	0.007	9.17	0.002
C18:2n-9	-	4.92 ± 0.99	6.69 ± 4.25	0.44 ± 0.44	1.73 ± 1.73	-	-	0.045	1.53	0.265
C18:2n-6	17.92	7.61 ± 1.23	8.70 ± 5.39	5.29 ± 2.76	6.42 ± 3.34	3.40 ± 1.97	0.77 ± 0.77	0.041	2.06	0.155
C18:3n-6	-	1.24 ± 0.72	-	-	1.94 ± 1.94	-	0.80 ± 0.80	0.017	0.54	0.746
C18:3n-3	1.11	-	-	3.85 ± 3.85	8.22 ± 5.12	2.98 ± 2.98	-	0.054	1.00	0.463
C18:4n-3	-	-	-	-	-	-	1.59 ± 1.59	0.014	1.00	0.500
C20:1n-9	-	-	3.36 ± 3.36	0.75 ± 0.75	-	1.39 ± 1.39	-	0.033	0.63	0.683
C20:2n-6	-	-	-	-	-	-	-	-	-	-
C20:3n-6	0.18	-	4.49 ± 4 .49	-	-	-	-	0.040	0.81	0.567
C20:4n-6	4.65	-	-	0.74 ± 0.74	-	0.48 ± 0.48	-	0.007	0.81	0.571
C20:4n-3	1.05	-	-	-	-	-	-	-	-	-
C20:5n-3	11.17	1.20 ± 1.20	2.15 ± 2.15	1.02 ± 1.02	-	2.19 ± 1.27	6.13 ± 2.10	0.034	2.54	0.164
C22:5n-3	0.72	-	0.42 ± 0.42	0.56 ± 0.56	-	-	2.27 ± 1.65	0.012	2.02	0.161
C22:6n-3	12.88	3.66 ± 2.67^{b}	9.29 ± 3.23^{b}	2.70 ± 2.70^{b}	4.75 ± 2.98^{b}	28.82 ± 4.00^{a}	27.97 ± 4.37^{a}	0.073	12.96	0.007
Totals										
Saturates	37.88	41.68 ± 1.77	40.22 ± 6.33	38.25 ± 8.20	35.90 ± 4.74	33.28 ± 2.27	46.38 ± 3.03	0.086	1.48	0.279
Monoenoics	11.85	38.33 ± 5.01^{a}	28.04 ± 12.03^{ab}	47.13 ± 4.21^{a}	41.04 ± 3.86^{a}	27.53 ± 3.26^{ab}	8.72 ± 1.23^{b}	0.131	4.58	0.020
(n-6)	22.76	8.85 ± 1.75	13.19 ± 4.81	6.03 ± 3.02	8.36 ± 4.93	3.89 ± 2.32	1.57 ± 1.57	0.068	1.50	0.273
(n-3)	2.16	-	-	3.85 ± 3.85	8.22 ± 5.12	2.98 ± 2.98	2.79 ± 1.48	0.056	0.82	0.563
(n-3)HUFA[4]	24.78	4.86 ± 3.84^{b}	11.86 ± 4.83^{b}	4.28 ± 4.28^{b}	4.75 ± 2.98^{b}	31.01 ± 3.38^{a}	36.37 ± 1.58^{a}	0.084	11.62	0.001
Others[5]	0.57	6.29 ± 1.27	6.69 ± 4.25	0.44 ± 0.44	1.73 ± 1.73	1.31 ± 1.31	4.77 ± 1.14	0.049	1.03	0.453

[1] Means of four samples using raw data, * three samples. (MSE)$^{1/2}$ values were calculated after arcsin transformation of data.

[2] Means with the same letters in the same row are not significantly different (P > 0.05).

[3] A dash (-) means not detected.

[4] Highly unsaturated fatty acids of the (n-3) series with five or more double bonds.

[5] Include 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-4, 18:3n-4, 18:4n-1.

Table 11. Fatty acid composition (% of total fatty acids) of liver polar lipid in red drum fingerlings fed experimental diets for nine weeks[1,2,3]. Anova with 5 and 12 df was used to test differences among means.

Fatty acid	Initial	Diet						(MSE)$^{1/4}$	F value	Pr > F
		LF	N6	N3	N6+3	H3	MFO			
C14:0	1.08	0.45 ± 0.27^{b}	0.45 ± 0.20^{b}	0.53 ± 0.19^{b}	1.32 ± 0.43^{b}	0.16 ± 0.16^{b}	2.99 ± 0.91^{a}	0.009	5.57	0.007
C16:0	30.71	20.08 ± 1.70^{b}	15.19 ± 0.65^{bc}	14.35 ± 0.92^{c}	17.95 ± 2.24^{bc}	19.19 ± 1.45^{bc}	27.50 ± 2.51^{a}	0.032	9.11	0.001
C16:1n-7	2.23	15.34 ± 2.88^{a}	4.36 ± 0.46^{b}	4.53 ± 0.40^{b}	6.18 ± 0.86^{b}	3.37 ± 0.84^{b}	5.09 ± 0.64^{b}	0.030	9.17	0.001
C18:0	6.09	7.14 ± 0.75^{b}	5.45 ± 0.18^{c}	4.83 ± 0.30^{c}	6.02 ± 0.44^{bc}	5.80 ± 0.25^{bc}	9.34 ± 0.61^{a}	0.010	11.32	0.000
C18:1n-9	8.53	22.51 ± 1.68^{d}	38.54 ± 2.54^{ab}	42.63 ± 2.46^{a}	30.83 ± 1.52^{c}	32.93 ± 1.72^{bc}	4.55 ± 0.95^{e}	0.045	40.11	0.000
C18:1n-7	1.09	3.22 ± 0.27^{a}	-	0.18 ± 0.18^{b}	0.53 ± 0.53^{b}	-	1.15 ± 0.66^{b}	0.008	8.60	0.001
C18:2n-9	-	4.88 ± 0.29^{a}	5.18 ± 0.17^{a}	5.32 ± 0.88^{a}	4.83 ± 0.13^{a}	0.85 ± 0.49^{b}	-	0.006	54.51	0.000
C18:2n-6	17.92	9.06 ± 0.57^{ab}	12.56 ± 2.58^{a}	7.51 ± 2.93^{ab}	9.76 ± 0.83^{ab}	4.52 ± 0.74^{bc}	0.46 ± 0.46^{c}	0.034	6.27	0.004
C18:3n-6	-	3.25 ± 0.62^{a}	2.41 ± 0.82^{ab}	1.20 ± 0.89^{b}	2.42 ± 0.13^{ab}	-	-	0.009	8.59	0.001
C18:3n-3	1.11	-	1.71 ± 1.71^{b}	6.24 ± 2.21^{a}	3.25 ± 0.35^{b}	-	-	0.019	6.90	0.003
C18:4n-3	-	-	0.42 ± 0.42^{b}	1.59 ± 0.55^{a}	0.64 ± 0.43^{ab}	-	0.29 ± 0.29^{b}	0.007	2.99	0.056
C20:1n-9	-	1.01 ± 1.01	1.79 ± 0.13	1.73 ± 0.60	1.38 ± 0.48	1.26 ± 0.74	-	0.013	1.08	0.418
C20:2n-6	-	-	0.41 ± 0.25	-	0.39 ± 0.39	-	-	0.004	1.05	0.431
C20:3n-6	0.18	3.10 ± 2.61	0.74 ± 0.36	-	-	-	-	0.019	1.67	0.217
C20:4n-6	4.65	-	0.92 ± 0.39	0.39 ± 0.39	0.91 ± 0.59	0.19 ± 0.19	0.58 ± 0.58	0.009	0.76	0.596
C20:4n-3	1.05	0.29 ± 0.29	-	-	-	-	0.33 ± 0.33	0.004	0.80	0.569
C20:5n-3	11.17	2.68 ± 1.31^{b}	1.27 ± 0.21^{bc}	1.34 ± 0.50^{bc}	1.68 ± 0.58^{bc}	0.24 ± 0.24^{c}	11.89 ± 0.53^{a}	0.014	40.26	0.000
C22:5n-3	0.72	1.54 ± 1.54	-	0.40 ± 0.40	0.33 ± 0.33	-	3.34 ± 2.01	0.016	2.80	0.067
C22:6n-3	12.88	5.47 ± 0.52^{b}	8.60 ± 1.06^{b}	7.21 ± 0.90^{b}	11.57 ± 1.07^{b}	31.35 ± 1.66^{a}	31.63 ± 2.66^{a}	0.037	45.21	0.000
Totals										
Saturates	37.88	27.67 ± 1.16^{b}	21.10 ± 0.45^{c}	19.72 ± 0.78^{c}	25.30 ± 2.67^{bc}	25.15 ± 1.22^{bc}	39.83 ± 3.84^{a}	0.039	15.09	0.000
Monoenoics	11.85	42.07 ± 2.27^{ab}	44.69 ± 2.29^{ab}	49.08 ± 2.72^{a}	38.92 ± 1.27^{b}	37.56 ± 1.94^{b}	10.79 ± 1.21^{c}	0.049	33.42	0.000
(n-6)	22.76	15.41 ± 2.27^{a}	17.03 ± 4.04^{a}	9.10 ± 3.67^{ab}	13.48 ± 1.17^{a}	4.71 ± 0.68^{b}	1.05 ± 1.05^{b}	0.050	6.40	0.004
(n-3)	2.16	0.29 ± 0.29^{b}	2.13 ± 2.13^{b}	7.83 ± 2.75^{a}	3.89 ± 0.57^{b}	-	0.62 ± 0.62^{b}	0.025	5.82	0.006
(n-3)HUFA[4]	24.78	9.69 ± 2.51^{c}	9.87 ± 1.25^{c}	8.95 ± 1.31^{c}	13.58 ± 1.56^{c}	31.59 ± 1.86^{b}	46.87 ± 3.46^{a}	0.053	38.85	0.000
Others[5]	0.57	4.88 ± 0.29^{a}	5.18 ± 0.17^{a}	5.32 ± 0.88^{a}	4.83 ± 0.12^{a}	0.98 ± 0.57^{b}	0.84 ± 0.84^{b}	0.010	18.80	0.000

[1] Means of four samples using raw data. (MSE)$^{1/4}$ values were calculated after arcsin transformation of data.

[2] Means with the same letters in the same row are not significantly different (P > 0.05).

[3] A dash (-) means not detected.

[4] Highly unsaturated fatty acids of the (n-3) series with five or more double bonds.

[5] Include 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-4, 18:3n-4, 18:4n-1.

Table 12. Fatty acid composition (% of total fatty acids) of liver nonpolar lipid in red drum fingerlings fed experimental diets for four weeks[1,2,3]. Anova with 5 and 10 df was used to test differences among means.

Fatty acid	Initial	Diet						(MSE)[½]	F value	Pr > F
		LF	N6	N3*	N6+3*	H3	MFO			
C14:0	3.50	2.50 ± 0.39^{b}	1.35 ± 0.52^{b}	2.48 ± 1.08^{b}	1.09 ± 0.57^{b}	0.97 ± 0.41^{b}	4.47 ± 0.34^{a}	0.011	5.50	0.042
C16:0	19.67	23.77 ± 2.02^{a}	15.12 ± 1.08^{bc}	20.59 ± 5.06^{ab}	16.44 ± 2.56^{b}	10.13 ± 1.67^{c}	20.61 ± 1.47^{ab}	0.036	6.97	0.005
C16:1n-7	5.69	17.26 ± 2.35^{a}	5.35 ± 0.68^{bc}	6.92 ± 2.79^{bc}	5.32 ± 0.69^{bc}	3.78 ± 1.38^{c}	10.38 ± 0.44^{b}	0.034	20.70	0.002
C18:0	6.53	9.00 ± 0.84^{a}	6.27 ± 1.04^{bc}	7.36 ± 1.32^{ab}	5.46 ± 0.63^{bcd}	3.56 ± 0.50^{d}	4.88 ± 0.33^{cd}	0.012	9.70	0.001
C18:1n-9	19.01	28.08 ± 1.43^{ab}	35.87 ± 6.45^{a}	28.91 ± 16.40^{ab}	50.28 ± 3.07^{a}	42.25 ± 9.17^{a}	9.57 ± 0.65^{b}	0.141	4.13	0.027
C18:1n-7	3.77	4.29 ± 0.45^{a}	2.87 ± 1.68^{ab}	1.20 ± 0.65^{b}	0.54 ± 0.54^{b}	1.59 ± 0.94^{ab}	3.98 ± 0.26^{a}	0.015	3.54	0.042
C18:2n-9	0.58	5.71 ± 1.02^{a}	3.37 ± 0.94^{abc}	2.12 ± 0.55^{bc}	4.21 ± 0.32^{ab}	1.67 ± 0.68^{bc}	0.95 ± 0.95^{c}	0.017	4.33	0.023
C18:2n-6	15.18	4.33 ± 0.96^{b}	13.89 ± 3.78^{a}	6.67 ± 1.93^{b}	8.14 ± 1.72^{ab}	4.22 ± 0.86^{b}	2.46 ± 0.83^{b}	0.040	4.25	0.025
C18:3n-6	-	0.83 ± 0.32	1.09 ± 0.45	0.34 ± 0.34	0.79 ± 0.40	2.00 ± 2.00	0.17 ± 0.17	0.019	0.47	0.788
C18:3n-3	5.37	-	1.43 ± 1.43	10.66 ± 5.10	2.55 ± 1.62	1.35 ± 1.01	1.58 ± 0.53	0.041	2.04	0.157
C18:4n-3	0.58	-	-	0.72 ± 0.40^{ab}	0.38 ± 0.38^{ab}	0.15 ± 0.15^{b}	1.40 ± 0.48^{a}	0.006	3.35	0.049
C20:1n-9	2.90	1.23 ± 0.29	2.39 ± 0.87	3.39 ± 1.81	0.72 ± 0.72	1.88 ± 0.79	1.55 ± 0.12	0.018	0.68	0.651
C20:2n-6	-	-	-	-	-	-	-	-	-	-
C20:3n-6	7.25	1.05 ± 0.41^{a}	0.72 ± 0.72^{a}	-	0.82 ± 0.82^{a}	0.38 ± 0.38^{a}	0.38 ± 0.38^{a}	0.010	0.39	0.844
C20:4n-6	-	-	0.55 ± 0.55^{ab}	0.39 ± 0.39^{ab}	-	0.19 ± 0.19^{b}	1.37 ± 0.34^{a}	0.006	2.49	0.103
C20:4n-3	1.18	-	-	-	-	-	1.70 ± 0.07^{a}	0.001	465.61	0.000
C20:5n-3	1.79	0.57 ± 0.39^{a}	0.63 ± 0.39^{a}	1.37 ± 0.26^{a}	-	8.51 ± 6.85^{a}	11.15 ± 0.39^{a}	0.066	2.04	0.158
C22:5n-3	-	-	0.44 ± 0.44^{b}	0.80 ± 0.80^{b}	-	0.38 ± 0.38^{b}	6.96 ± 0.53^{a}	0.009	33.92	0.000
C22:6n-3	1.97	0.83 ± 0.56^{b}	4.11 ± 2.30^{b}	5.07 ± 2.90^{ab}	2.33 ± 2.33^{b}	15.51 ± 5.27^{a}	11.45 ± 1.93^{ab}	0.062	3.37	0.048
Totals										
Saturates	29.69	35.27 ± 2.47^{a}	22.74 ± 2.11^{b}	30.44 ± 7.09^{ab}	22.99 ± 2.47^{b}	14.65 ± 2.08^{c}	29.96 ± 1.57^{ab}	0.048	9.30	0.002
Monoenoics	31.38	50.86 ± 3.27	46.49 ± 6.25	40.43 ± 13.10	56.83 ± 3.48	49.68 ± 11.17	25.47 ± 1.20	0.158	2.24	0.131
(n-6)	22.43	6.21 ± 0.83^{b}	16.25 ± 3.17^{a}	7.40 ± 2.43^{b}	9.76 ± 1.46^{b}	6.79 ± 1.23^{b}	4.38 ± 1.05^{b}	0.039	4.85	0.016
(n-3)	7.13	-	1.43 ± 1.43	11.69 ± 5.78	3.88 ± 2.89	1.77 ± 1.26	4.68 ± 0.11	0.049	1.80	0.201
(n-3)HUFA[4]	3.76	1.40 ± 0.95^{c}	5.18 ± 2.95^{bc}	7.24 ± 3.94^{abc}	2.33 ± 2.33^{bc}	24.40 ± 12.02^{ab}	29.57 ± 2.50^{a}	0.132	3.45	0.045
Others[5]	5.61	6.26 ± 0.95	7.91 ± 3.76	2.80 ± 0.23	4.21 ± 0.32	2.72 ± 0.95	5.94 ± 1.09	0.040	0.95	0.491

[1] Means of four samples using raw data, *three samples. (MSE)[½] values were calculated after arcsin transformation of data.

[2] Means with the same letters in the same row are not significantly different (P > 0.05).

[3] A dash (-) means not detected.

[4] Highly unsaturated fatty acids of the (n-3) series with five or more double bonds.

[5] Include 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-4, 18:3n-4, 18:4n-1.

Table 13. Fatty acid composition (% total fatty acid) of nonpolar liver lipid in red drum fingerlings fed experimental diets for nine weeks[1,2,3]. Anova with 5 and 12 df was used to test differences among means.

Fatty acid	Initial	Diet						(MSE)[4]	F value	Pr > F
		LF	N6	N3	N6+3	H3	MFO			
C14:0	3.50	1.77 ± 0.57^{b}	0.61 ± 0.23^{b}	0.74 ± 0.26^{b}	1.14 ± 0.08^{b}	0.66 ± 0.59^{b}	5.49 ± 0.84^{a}	0.008	20.30	0.000
C16:0	19.67	19.83 ± 1.12^{a}	12.66 ± 2.44^{b}	9.87 ± 1.15^{b}	13.81 ± 2.11^{b}	12.74 ± 2.26^{b}	24.39 ± 1.46^{a}	0.039	7.94	0.002
C16:1n-7	5.69	24.17 ± 1.00^{a}	6.38 ± 1.07^{c}	5.71 ± 0.35^{c}	5.32 ± 1.57^{c}	5.48 ± 1.61^{c}	12.54 ± 0.49^{b}	0.024	39.70	0.000
C18:0	6.53	7.27 ± 0.66^{a}	3.72 ± 0.63^{c}	2.58 ± 0.34^{c}	4.56 ± 0.78^{bc}	4.11 ± 0.77^{c}	6.26 ± 0.45^{ab}	0.013	7.33	0.002
C18:1n-9	19.01	31.88 ± 1.85^{c}	57.50 ± 4.10^{ab}	61.14 ± 4.22^{a}	32.68 ± 10.93^{c}	44.12 ± 3.25^{bc}	13.76 ± 0.62^{d}	0.116	11.55	0.000
C18:1n-7	3.77	3.00 ± 1.01	-	-	16.01 ± 15.39	0.67 ± 0.67	3.73 ± 0.21	0.137	0.93	0.495
C18:2n-9	0.58	6.51 ± 0.68^{a}	6.25 ± 0.21^{a}	6.35 ± 0.73^{a}	7.33 ± 0.65^{a}	2.14 ± 0.25^{b}	-	0.010	36.77	0.000
C18:2n-6	15.18	1.78 ± 0.17^{c}	7.25 ± 0.75^{a}	1.99 ± 0.32^{c}	5.80 ± 0.60^{b}	2.98 ± 0.35^{c}	2.89 ± 0.02^{c}	0.009	23.49	0.000
C18:3n-6	-	0.68 ± 0.10	1.86 ± 0.04	0.87 ± 0.32	4.36 ± 2.69	0.23 ± 0.23	0.28 ± 0.10	0.023	1.91	0.166
C18:3n-3	5.37	-	-	3.74 ± 0.20^{a}	2.05 ± 0.27^{b}	0.98 ± 0.98^{bc}	0.84 ± 0.02^{bc}	0.008	11.43	0.000
C18:4n-3	0.58	-	-	1.45 ± 0.47^{a}	0.42 ± 0.26^{b}	1.57 ± 0.93^{a}	1.95 ± 0.08^{a}	0.004	16.75	0.000
C20:1n-9	2.90	0.61 ± 0.07^{c}	1.95 ± 0.14^{abc}	2.32 ± 0.32^{ab}	1.54 ± 0.57^{abc}	3.13 ± 0.85^{a}	0.83 ± 0.29^{bc}	0.010	3.62	0.032
C20:2n-6	-	-	0.21 ± 0.12	-	-	-	-	0.001	1.92	0.165
C20:3n-6	7.25	0.03 ± 0.03	-	-	0.21 ± 0.21	0.60 ± 0.35	0.17 ± 0.10	0.004	1.31	0.323
C20:4n-6	-	-	-	-	-	0.54 ± 0.54	0.71 ± 0.12	0.005	2.08	0.139
C20:4n-3	1.18	-	-	-	0.52 ± 0.31^{b}	0.38 ± 0.38^{b}	1.51 ± 0.04^{a}	0.004	7.36	0.002
C20:5n-3	1.79	-	0.09 ± 0.09^{b}	0.09 ± 0.09^{b}	1.00 ± 0.70^{b}	2.05 ± 1.32^{b}	9.57 ± 0.45^{a}	0.013	35.23	0.000
C22:5n-3	-	-	-	1.69 ± 1.51^{b}	-	0.71 ± 0.71^{b}	4.51 ± 0.09^{a}	0.014	6.46	0.004
C22:6n-3	1.97	0.23 ± 0.09^{b}	0.50 ± 0.24^{b}	0.65 ± 0.33^{b}	2.89 ± 1.13^{b}	14.92 ± 3.50^{a}	4.42 ± 0.41^{b}	0.028	16.65	0.000
Totals										
Saturates	29.69	29.09 ± 0.60^{a}	16.98 ± 3.06^{b}	13.20 ± 1.46^{b}	19.52 ± 2.78^{b}	17.50 ± 3.24^{b}	36.14 ± 1.44^{a}	0.051	12.49	0.000
Monoenoics	31.38	59.68 ± 1.64^{bc}	65.97 ± 3.05^{ab}	69.30 ± 3.93^{a}	55.55 ± 4.71^{c}	53.39 ± 2.28^{c}	30.86 ± 1.09^{d}	0.073	19.53	0.000
(n-6)	22.43	2.50 ± 0.22^{b}	9.32 ± 0.69^{a}	2.86 ± 0.64^{b}	10.38 ± 3.04^{a}	4.34 ± 0.79^{b}	4.06 ± 0.27^{b}	0.030	5.15	0.009
(n-3)	7.13	-	-	5.19 ± 0.38^{a}	2.98 ± 0.45^{b}	2.93 ± 0.98^{b}	4.31 ± 0.06^{ab}	0.010	20.26	0.000
(n-3)HUFA[4]	3.76	0.23 ± 0.09^{c}	0.59 ± 0.26^{bc}	2.43 ± 1.27^{bc}	3.89 ± 1.30^{b}	17.68 ± 1.91^{a}	18.50 ± 0.40^{a}	0.021	65.58	0.000
Others[5]	5.61	8.50 ± 1.59	7.15 ± 0.57	7.03 ± 0.83	7.69 ± 0.79	4.15 ± 1.09	6.14 ± 0.95	0.020	2.39	0.101

[1] Means of four samples using raw data. (MSE)[4] values were calculated after arcsin transformation of data.

[2] Means with the same letters in the same row are not significantly different (P > 0.05).

[3] A dash (-) means not detected.

[4] Highly unsaturated fatty acids of the (n-3) series with five or more double bonds.

[5] Include 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-4, 18:3n-4, 18:4n-1.

Monoenoic fatty acids (MFAs)

Muscle polar lipid: Monoenoic fatty acids in the muscle polar lipid of fish fed LF and FAEEs increased ($P < 0.05$) during the feeding period, while fish fed MFO decreased levels of MFAs during the same period (Figure 2). In both week four and week nine, fish fed MFO had lower ($P < 0.05$) MFA levels than fish fed LF and FAEEs, and there was no difference ($P > 0.05$) among fish fed LF and FAEEs (Tables 6, 7). In week four, MFAs ranged from 15.5 to 34.2%. Oleic acid (18:1n-9), the predominant fatty acid in the FAEE diets, was higher ($P < 0.05$) in fish fed those diets than in fish fed MFO, but did not differ ($P > 0.05$) among fish fed FAEEs or the LF diet. Oleic acid was 2 - 3 times higher in fish fed FAEE diets than in fish fed MFO (Table 6). In week nine, MFAs ranged from 15.0 to 40.8%. Oleic acid was higher ($P < 0.05$) in fish fed FAEEs than in fish fed LF, while fish fed LF were higher ($P < 0.05$) in oleic acid than those fed MFO. Oleic acid in fish fed FAEEs was about four times higher than in fish fed MFO (Table 7). Palmitoleic acid (16:1n-7) was higher ($P < 0.05$) in fish fed LF than in fish fed FAEE and MFO diets in both week four and week nine (Table 6 , 7).

Muscle nonpolar lipid: There was no significant change in total MFA levels in muscle nonpolar lipid of fish fed all six diets during the course of the study (Figure 3). Fish fed the six diets did not differ ($P > 0.05$) in total MFAs in week four or week nine. MFA concentrations ranged from 23.9 to 42.8% in week four (Table 8), and from 31.5 to 43.2% in week nine (Table 9). In week four, 18:1n-9 was significantly higher in fish fed N6, N3, and N6+3 than in fish fed MFO. Fish fed LF, and H3 did

not differ ($P > 0.05$) in 18:1n-9 content from fish fed MFO, N6, N3, or N6+3 (Table 8). Levels of 16:1n-7 were higher ($P < 0.05$) in fish fed LF and MFO than in fish fed FAEEs. In week nine, 18:1n-9 was significantly higher in fish fed FAEEs than in fish fed MFO (Table 9). Fish fed LF did not differ ($P > 0.05$) in 18:1n-9 content from fish fed MFO or FAEEs. Fish fed LF had higher ($P < 0.05$) 16:1n-7 concentrations than fish fed FAEEs, but they did not differ ($P > 0.05$) from fish fed MFO.

Liver polar lipid: Total MFAs in the liver polar lipid of fish fed LF and FAEEs increased during the nine-week feeding period (Figure 4). In fish fed MFO there was no significant change in MFA levels. In week four, total MFAs ranged from 8.7 to 47.0% (Table 10). There was no difference ($P > 0.05$) in total MFAs among fish fed LF and FAEEs. Fish fed MFO had lower ($P < 0.05$) total MFAs than fish fed LF, N3, and N6+3. MFA levels in fish fed MFO did not differ ($P > 0.05$) from those in fish fed N6 and H3. Oleic acid (18:1n-9) was lowest in fish fed MFO; less than 15% of the oleic acid levels in fish fed FAEEs. Oleic acid concentration in fish fed LF did not differ ($P > 0.05$) from that in fish fed N6, and H3, but was lower ($P < 0.05$) than in fish fed N3, and N6+3. Palmitoleic acid (16:1n-9) was higher ($P < 0.05$) in fish fed LF than in fish fed FAEEs, but did not differ ($P > 0.05$) from 16:1n-7 concentration in fish fed MFO (Table 10).

In week nine, total MFAs ranged from 10.8 to 49.1% (Table 11). Fish fed N3 had higher ($P < 0.05$) total MFAs than fish fed N6+3, H3, or MFO, but did not differ ($P < 0.05$) from MFA levels in fish fed LF and N6. Total MFAs in fish fed MFO were significantly lower than in those fed LF and FAEEs. Oleic acid was significantly

higher in fish fed FAEEs than in fish fed LF or MFO. Oleic acid level in fish fed MFO was lower ($P < 0.05$) than in fish fed LF and was less than 20% of 18:1n-9 concentrations in fish fed FAEEs. Palmitoleic acid was highest ($P < 0.05$) in fish fed LF. It was not significantly different among fish fed MFO and FAEEs.

Liver nonpolar lipid: Total MFAs in liver nonpolar lipid of fish fed LF, N6, N3, and N6+3 increased significantly by the end of the feeding period, but in fish fed H3, and MFO there was no significant change (Figure 5). In week four, total MFAs ranged from 25.5 to 56.8% (Table 12). There was no significant difference in MFA concentrations among fish fed all diets. Oleic acid level did not differ ($P > 0.05$) among fish fed LF and FAEEs. Fish fed MFO had lower ($P < 0.05$) 18:1n-9 levels than fish fed N6, N6+3, and H3, but did not differ ($P > 0.05$) from those fed LF and N3. Fish fed LF had the highest level of 16:1n-7. There was no significant difference in the level of 16:1n-7 among fish fed FAEEs or MFO, with one exception; 16:1n-7 in fish fed H3 was lower ($P < 0.05$) than in those fed MFO (Table 15). In week nine, total MFAs ranged from 30.9 to 69.3% (Table 16). MFAs were significantly lower in fish fed MFO than in fish fed LF and FAEEs. Fish fed LF had lower ($P < 0.05$) MFAs than those fed N3, but did not differ ($P > 0.05$) from those fed N6, N6+3, or H3. Fish fed N6 and N3 had higher ($P < 0.05$) 18:1n-9 concentrations than those fed LF, N6+3, or MFO. Oleic acid levels did not differ ($P > 0.05$) among fish fed N6, N3, and H3, or among those fed N6+3 and H3. Fish fed MFO had the lowest level of 18:1n-9 in nonpolar liver lipid. Fish fed LF had the highest level of 16:1n-7, followed by those fed MFO. There was no significant difference in 16:1n-7

concentration among fish fed FAEEs. Other MFAs (18:1n-7 and 20:1n-9), if detected, were present in small amounts in all tissue lipids. Their occurrence did not appear to be diet related.

Polyunsaturated fatty acids of the linoleic family (n-6)

Muscle polar lipid: In muscle polar lipid, the level of total n-6 polyunsaturated fatty aids (PUFAs) decreased ($P < 0.05$) in fish fed N3, H3, and MFO during the course of the study, but not in fish fed LF, N6, and N6+3 (Figure 2). In week four, total n-6 PUFAs ranged from 7.4 to 23.7% (Table 6). Fish fed MFO had the lowest level n-6 PUFAs. Fish fed N6 and N6+3 had higher ($P < 0.05$) n-6 PUFAs than those fed N3, H3, and MFO, but did not differ ($P > 0.05$) in n-6 PUFA levels from fish fed LF.

In week nine, total n-6 PUFAs ranged from 8.3 to 21.8% (Table 7). Fish fed N6 and N6+3 had significantly higher n-6 PUFA levels than those fed N3, H3, and MFO, but did not differ ($P > 0.05$) in n-6 PUFA concentration from fish fed LF. There was no significant difference in n-6 PUFA content of fish fed N3, H3, and MFO. In week four, linoleic acid (18:2n-6) content of fish fed N6 did not differ ($P > 0.05$) from that of fish fed N6+3, but was higher ($P < 0.05$) than 18:2n-6 content of fish fed all other diets without 18:2n-6 supplements (LF, N3, H3, and MFO) (Table 6). The linoleic acid level in fish fed N6+3 did not differ ($P > 0.05$) from that of fish fed LF, but was higher ($P < 0.05$) than 18:2n-6 levels in fish fed N3, H3, and MFO. γ-Linolenic acid (18:3n-6) was detected in fish fed LF, N6, and N6+3, but not in those fed N3, H3 and MFO. γ-Linolenic acid was lower ($P < 0.05$) in fish fed N6 than in

those fed LF, but did not differ ($P > 0.05$) from 18:3n-6 in fish fed N6+3. Eicosadienoic acid (20:2n-6) was detected only in fish fed N6 and di-homo-γ-linolenic acid (20:3n-6) was detected only in fish fed N6+3. Arachidonic acid (20:4n-6) was detected in fish fed all six diets. There was no significant difference in 20:4n-6 levels among treatment groups (Table 6). In week nine, the level of 18:2n-6 in fish fed N6 and N6+3 was higher ($P < 0.05$) than in those fed N3, H3, and MFO, but did not differ ($P > 0.05$) from 18:2n-6 in fish fed LF (Table 7). The level of 18:3n-6 in fish fed N6+3 was lower ($P < 0.05$) than in those fed LF but did not differ ($P > 0.05$) from fish fed N6 and MFO. γ-Linolenic acid was not detected in fish fed N3 and H3. Levels of 20:2n-6 did not differ ($P < 0.05$) among fish fed LF, N6, N6+3 and MFO. It was not detected in fish fed N3 and H3. Di-homo-γ-linolenic acid (20:3n-6) levels did not differ ($P > 0.05$) among fish fed LF, N6+3, and MFO. It was not detected in fish fed N6, N3 and H3. Arachidonic acid (20:4n-6) was detected in fish fed all six diets. Arachidonic acid levels in fish fed N6, N3, and N6+3 were higher ($P < 0.05$) than in fish fed LF and H3, but did not differ ($P > 0.05$) from 20:4n-6 levels in fish fed MFO. The arachidonic acid content of fish fed H3 did not differ ($P < 0.05$) from that of fish fed LF and MFO (Table 7).

Muscle nonpolar lipid: Total n-6 PUFAs in muscle nonpolar lipid of fish fed MFO decreased significantly by the end of the feeding period (Figure 3). Levels of n-6 PUFAs in fish fed LF and FAEEs did not differ ($P > 0.05$). In week four, total n-6 PUFAs ranged from 4.4 to 22.8% (Table 8). There was no significant difference in total n-6 PUFAs among fish fed LF, N6, N3, N6+3 and MFO. Levels of n-6 PUFAs

were higher ($P < 0.05$) in fish fed H3 than in fish fed LF, N6+3, N3, and MFO, but did not differ ($P > 0.05$) from levels in fish fed N6. Linoleic acid level in fish fed N6 was higher ($P < 0.05$) than in fish fed LF, H3, and MFO, but not did differ ($P > 0.05$) from 18:2n-6 levels in fish fed N3 and N6+3. Fish fed N6+3 contained higher ($P < 0.05$) concentrations of 18:2n-6 than those fed MFO, but did not differ ($P > 0.05$) from fish fed LF, N3, and H3. γ-Linolenic acid (18:3n-6) was detected in fish fed LF, N3, and N6+3, but not in fish fed N6, H3, and MFO. Di-homo-γ-linolenic acid (20:3n-6) was detected only in fish fed H3; 20:2n-6 was not detected in any treatment groups. Arachidonic acid (20:4n-6) levels in fish fed N3 and MFO were lower ($P < 0.05$) than in those fed H3. Arachidonic acid was not detected in fish fed LF, N6, and N6+3 (Table 8).

In week nine, total n-6 PUFAs ranged from 4.4 to 16.4% (Table 9). There was no difference ($P > 0.05$) in total n-6 PUFAs among fish fed the six diets. Levels of 18:2n-6 in fish fed N6, N6+3, and H3 were higher ($P < 0.05$) than in fish fed MFO, but did not differ ($P > 0.05$) from levels in fish fed LF and N3. γ-Linolenic acid (18:3n-6) and 20:2n-6 were detected only in fish fed N3 and MFO, respectively, while 20:3n-6 was detected in fish fed N6, N3, and H3. Arachidonic acid (20:4n-6) levels did not differ ($P > 0.05$) among fish fed N3, N6+3, H3, and MFO; (20:4n-6) was not detected in fish fed LF and N6 (Table 9).

Liver polar lipid: In liver polar lipid, concentrations of n-6 PUFAs decreased ($P < 0.05$) in fish fed diets without an 18:2n-6 supplement (LF, N3, H3, and MFO), but there was no ($P > 0.05$) difference in n-6 PUFA levels among fish fed N6 and

N6+3 diets (Figure 4). In week four, total n-6 PUFAs ranged from 1.6 to 12.1% (Table 10). There was no difference ($P > 0.05$) in concentrations of total n-6 PUFAs among fish fed the six diets. Linoleic acid levels were not different ($P > 0.05$) among fish fed all diets. Levels of 18:3n-6 were not different ($P > 0.05$) among fish fed LF, N6+3, and MFO; 18:3n-6 was not detected in fish fed N6, N3, and H3. Di-homo-γ-linolenic acid (20:3n-6) was detected only in fish fed N6. Arachidonic acid (20:4n-6) level did not differ ($P > 0.05$) among fish fed N3 and H3; 20:4n-6 was not detected in fish fed the other diets.

In week nine, total n-6 PUFAs ranged from 1.0 to 17.0% (Table 11). Fish fed LF, N6, and N6+3 had total n-6 PUFA concentrations higher ($P < 0.05$) than in fish fed H3 and MFO, but not different ($P > 0.05$) from those fed N3. Fish fed N6 had a higher ($P < 0.05$) 18:2n-6 level than those fed H3 and MFO, but did not differ ($P > 0.05$) in 18:2n-6 content from those fed LF, N3, and N6+3. γ-Linolenic acid (18:3n-6) was higher ($P < 0.05$) in fish fed LF than in those fed N3. Neither LF- nor N3-fed fish differed ($P < 0.05$) in 18:3n-6 content from those fed N6 or N6+3; 18:3n-6 was not detected in fish fed H3 and MFO. Fish fed N6 and N6+3 had detected levels of 20:2n-6 with no difference ($P > 0.05$) in concentration. Di-homo-γ-linolenic acid was detected only in fish fed LF and N6, with no significant difference in concentration between treatments. Arachidonic acid was detected in fish fed N6, N3, N6+3, H3, and MFO, but not in those fed LF. There was no significant difference in 20:4n-6 levels among fish fed the FAEE and MFO diets (Table 11).

Liver nonpolar lipid: Total n-6 PUFAs in liver nonpolar lipid decreased in fish fed the six diets (Figure 5). In week four, total n-6 PUFAs ranged from 4.4 to 16.3% and was highest in fish fed N6 (Table 12). There was no significant difference in n-6 PUFA concentrations in fish fed LF, N3, N6+3, H3, and MFO. The level of 18:2n-6 was higher ($P < 0.05$) in fish fed N6 than in those fed LF, N3, H3, and MFO, but did not differ ($P > 0.05$) from the level in fish fed N6+3. There was no significant difference in the level of 18:3n-6 in fish fed the six diets; 20:2n-6 was not detected in fish in any treatment group. Levels of 20:3n-6 were not significantly different in fish fed LF, N6, N6+3, H3, and MFO; 20:3n-6 was not detected in fish fed N3. Levels of arachidonic acid (20:4n-6) were not different ($P > 0.05$) among fish fed all diets. Arachidonic acid was not detected in fish fed LF and N6+3.

In week nine, n-6 PUFAs ranged from 2.5 to 10.4% (Table 13). There was no significant difference in n-6 PUFAs in fish fed N6 and N6+3. Both N6- and N6+3-fed fish contained higher ($P < 0.05$) levels of N6 PUFAs than fish fed the other diets. Linoleic acid (18:2n-6) was highest in fish fed N6, followed by those fed N6+3. There was no difference ($P > 0.05$) in the levels of 18:2n-6 in fish fed the other diets, all of which contained less 18:2n-6 than N6+3-fed fish did. Levels of γ-linoleic acid (18:3n-6) did not differ ($P > 0.05$) among fish fed all diets. Di-homo-γ-linoleic acid (20:2n-6) was detected only in fish fed N6. Detectable levels of 20:3n-6 were found in fish fed LF, N6+3, H3, and MFO, but not in fish fed N6 and N3. Arachidonic acid (20:4n-6) was detected only in fish fed H3 and MFO, and concentrations were not significantly different (Table 13).

Polyunsaturated fatty acids of the linolenic family (n-3 PUFAs with 3 or 4 double bonds)

Muscle polar lipid: Total n-3 PUFAs in the muscle polar lipid of fish fed N3, N6+3, and MFO increased significantly by the end of the feeding period (Figure 2). There was no change ($P > 0.05$) in the n-3 PUFA content of fish fed LF during the course of the study. There were no n-3 PUFAs detected in fish fed N6 and H3 at week four and week nine. In week four, n-3 PUFAs ranged from 1.2 to 6.0% and were highest ($P < 0.05$) in fish fed N3 (table 6). Fish fed N6+3 had higher ($P < 0.05$) n-3 PUFAs than those fed LF, but did not differ ($P > 0.05$) in n-3 PUFA content from those fed MFO.

In week nine, n-3 PUFAs ranged from 0.4 to 8.9% (Table 7). Fish fed N3 contained the highest ($P < 0.05$) concentration of n-3 PUFAs followed by those fed N6+3 ($P < 0.05$), MFO ($P < 0.05$), and LF ($P < 0.05$). In both week four and week nine, 18:3n-3 was highest ($P < 0.05$) in fish fed N3. Fish fed N6+3 were higher ($P < 0.05$) in 18:3n-3 than fish fed LF or MFO. In week four, 18:4n-3 was detected in fish fed LF, N6+3 and MFO; in week nine, it was detected only in fish fed N3 and MFO. In week four and week nine, 20:4n-3 was detected only in fish fed MFO.

Muscle nonpolar lipid: Total n-3 PUFAs in muscle nonpolar lipid of fish fed N3 and MFO increased significantly by the end of the feeding period, while in those fed N6, N6+3, and H3 there was no significant change in n-3 PUFA concentrations (Figure 3). N-3 PUFAs were not detected in fish fed LF during the study. In week

four, n-3 PUFAs ranged from 0.9 to 5.4% and were highest ($P < 0.05$) in fish fed N3, followed by those fed MFO ($P < 0.05$), and those fed N6+3 ($P < 0.05$) (Table 8). N-3 PUFAs were not detected in fish fed LF, N6, and H3. Linoleic acid (18:3n-3) was higher ($P < 0.05$) in fish fed N3 than in those fed N6+3; it was not detected in fish fed the diets that were not supplemented with 18:3n-3. Detectable levels of 18:4n-3 and 20:4n-3 were found only in fish fed MFO.

In week nine, n-3 PUFAs ranged from 0.7 to 6.3% (Table 9). There were no significant differences in the levels of n-3 PUFAs among fish fed all diets. Linolenic acid level 18:3n-3 was higher ($P < 0.05$) in fish fed N3 than in those fed N6+3 and MFO, but there was no difference ($P > 0.05$) in 18:3n-3 level of fish fed N6+3 and MFO. A detectable level of 18:4n-3 was found only in fish fed N6 and MFO. All fish, except those fed LF had detectable levels of 20:4n-3, but there were no differences ($P < 0.05$) in 20:4n-3 concentrations among fish fed the FAEE and MFO diets.

Liver polar lipid: Levels of total n-3 PUFAs in liver polar lipid of fish fed the six experimental diets did not change significantly during the feeding trial (Figure 4). In week four, n-3 PUFAs ranged from 2.8 to 8.2% (Table 10). N-3 PUFAs were not detected in fish fed LF and N6. There were no significant differences in n-3 PUFA content of fish fed the other diets. Linolenic acid (18:3n-3) was detected in fish fed N3, N6+3 and H3, and did not differ significantly in concentration among them. A detectable amount of 18:4n-3 was found only in fish fed MFO; 20:4n-3 was not detected in any treatment group.

In week nine, n-3 PUFAs ranged from 0.3 to 7.8% and were ($P < 0.05$) highest in fish fed N3 (Table 11). N-3 PUFAs did not differ significantly in concentration among fish fed LF, N6, N6+3, or MFO; they were not detected in fish fed H3. The level of 18:3n-3 was highest ($P < 0.05$) in fish fed N3; it was not detected in fish fed LF, H3, and MFO. There was no significant difference in 18:3n-3 content of fish fed N6 or N6+3. The level of 18:4n-3 was higher ($P < 0.05$) in fish fed N3 than in those fed N6 and MFO. Concentrations of 18:3n-4 did not differ ($P > 0.05$) in fish fed N3 and N6+3; it was not detected in fish fed LF and H3. A detectable amount of 20:4n-3 was found only in fish fed LF and MFO.

Liver nonpolar lipid: Total n-3 PUFAs decreased significantly by week nine in liver nonpolar lipid of fish fed LF and N6 (Figure 5). N-3 PUFAs were not detected in week four or week nine in fish fed LF. Fish fed N3, N6+3, H3, and MFO had n-3 PUFA concentrations that did not differ ($P > 0.05$) at week nine from initial concentrations (week 0). In week four, n-3 PUFAs ranged from 1.4 to 11.7% (Table 12). There was no significant difference in n-3 PUFA levels among fish fed all diets. Linolenic acid (18:3n-3) levels were not different ($P > 0.05$) among fish fed all diets. The level of 18:4n-3 in fish fed MFO was higher ($P < 0.05$) than in those fed H3, but did not differ ($P > 0.05$) from 18:4n-3 levels in fish fed N3 and N6+3; 18:4n-3 was not detected in fish fed LF and N6. A detectable level of 20:4n-3 was found only in fish fed MFO.

In week nine, n-3 PUFAs ranged from 2.9 to 5.2% and were higher ($P < 0.05$) in fish fed N3 than in those fed N6+3 or H3. N-3 PUFA concentrations did not differ

($P > 0.05$) among fish fed N6+3, H3, and MFO, nor among fish fed N3 and MFO. N-3 PUFA were not detected in fish fed LF and N6. Linolenic acid (18:3n-3) was highest ($P < 0.05$) in fish fed N3, but did not differ ($P > 0.05$) among fish fed N6+3, H3, and MFO. Levels of 18:4n-3 were higher ($P < 0.05$) in fish fed N3, H3, and MFO than in those fed N6+3. Detectable levels of 20:4n-3 were found in fish fed N6+3, H3, and MFO; 20:4n-3 concentration was higher ($P < 0.05$) in fish fed MFO than in those fed N6+3 and H3.

Highly unsaturated fatty acids of the linolenic family (n-3 HUFAs with 5 or 6 double bonds)

Muscle polar lipid: Total HUFAs in muscle polar lipid of fish fed LF, N6, and N3 decreased significantly during the feeding trial (Figure 2). HUFA concentrations increased significantly in fish fed MFO and were unchanged ($P > 0.05$) in fish fed N6+3 and H3. In week four, HUFAs ranged from 15.7 to 39.8% of muscle polar lipid and were highest ($P < 0.05$) in fish fed MFO (Table 6). Fish fed H3 had higher ($P < 0.05$) HUFA content than those fed LF, but did not differ ($P > 0.05$) in HUFA content from those fed N6, N3, and N6+3. Fish fed MFO had the highest ($P > 0.05$) eicosapentaenoic acid (EPA, 20:5n-3) and docosapentaenoic acid (22:5n-3) levels. MFO-fed fish also contained a higher ($P > 0.05$) level of docosahexaenoic acid (DHA, 22:6n-3) than fish fed LF, N6, N3, and N6+3, but did not differ ($P > 0.05$) in DHA content from fish fed H3, which contained supplemental 22:6n-3.

In week nine, HUFAs ranged from 14.2 to 44.5% (Table 7). HUFA concentrations were highest ($P < 0.05$) in fish fed MFO; concentrations did not differ

($P > 0.05$) among fish fed the other diets. EPA and 22:5n-3 levels were highest ($P < 0.05$) in fish fed MFO. Fish fed LF, N6, and N6+3 did not differ ($P > 0.05$) in EPA content, but all three were higher ($P < 0.05$) in EPA than fish fed H3. Fish fed N3 and H3 did not differ ($P > 0.05$) in 20:5n-3 content. Fish fed MFO were also higher ($P < 0.05$) in 22:6n-3 content than those fed LF, N6, N3, and N6+3, but did not differ ($P > 0.05$) in 22:6n-3 content from fish fed H3. Fish fed H3 were higher ($P < 0.05$) in 22:6n-3 than those fed LF, N3, and N6+3, but did not differ ($P > 0.05$) from fish fed N6.

Muscle nonpolar lipid: Total HUFAs in muscle nonpolar lipid of fish fed MFO increased significantly by the end of the feeding trial (Figure 3). There was no significant change in total HUFA level among fish fed the other diets. In week four, HUFAs ranged from 2.1 to 34.8% and were highest ($P < 0.05$) in fish fed MFO (Table 8). HUFA concentrations did not differ significantly among fish fed the other diets. Fish fed MFO had the highest ($P < 0.05$) level of 20:5n-3. Levels of 20:5n-3 did not differ ($P > 0.05$) among fish fed the other diets. A detectable amount of 22:5n-3 was found only in fish fed MFO. There was no significant difference in levels of 22:6n-3 among fish fed all diets.

In week nine, HUFAs ranged from 2.0 to 26.9% and were highest ($P < 0.05$) in fish fed MFO (Table 9). HUFA level was higher ($P < 0.05$) in fish fed H3 than in those fed LF, N6, and N3, but did not differ ($P < 0.05$) from fish fed N6+3. EPA (20:5n-3) was highest ($P < 0.05$) in fish fed MFO. There were no significant differences in EPA levels of fish fed the other diets. Detectable quantities of 22:5n-3

were found in fish fed LF, N6+3, H3, and MFO; 22:5n-3 was not detected in fish fed N6 and N3. There were no significant differences in 22:5n-3 content of fish that contained detectable quantities of the fatty acid. DHA (22:6n-3) was not detected in fish fed N6 and N6+3. In the remaining treatment groups, DHA was higher ($P < 0.05$) in fish fed H3 and MFO than in those fed LF and N3.

Liver polar lipid: Total HUFAs in liver polar lipid of fish fed MFO increased by the end of the feeding trial, while HUFA concentration decreased in fish fed LF (Figure 4). Fish fed the FAEE diets had no significant change in total HUFA concentrations at the end of the feeding trial. In week four, total HUFAs ranged from 4.3 to 36.4% (Table 10). Concentrations of total HUFAs did not differ ($P > 0.05$) between fish fed H3 and MFO, but both contained higher ($P < 0.05$) HUFA concentrations than fish fed the other diets. Fish fed LF, N6, N3, and N6+3 did not differ ($P > 0.05$) in HUFA content. EPA (20:5n-3) was not detected in fish fed N6+3, but was present in fish fed the other diets, where its concentration did not differ ($P > 0.05$) among treatment groups. Detectable levels of 22:5n-3 were found in fish fed N6, N3, and MFO, which did not differ significantly in 22:5n-3 concentration. Levels of 22:6n-3 were not different ($P > 0.05$) in fish fed H3 and MFO; but H3- and MFO-fed fish had higher ($P < 0.05$) 22:6n-3 levels than fish fed the other diets. Concentrations of 22:6n-3 did not differ ($P > 0.05$) among fish fed LF, N6, N3, and N6+3.

In week nine, total HUFAs ranged from 8.9 to 46.9% (Table 11). HUFA concentrations were highest ($P < 0.05$) in fish fed MFO followed by those fed H3 (P

< 0.05), followed by fish fed the rest of the diets ($P < 0.05$). HUFA levels did not differ ($P > 0.05$) among fish fed LF, N6, N3, and N6+3. EPA (20:5n-3) concentrations were highest ($P < 0.05$) in fish fed MFO, followed by those fed LF ($P < 0.05$). EPA in fish fed LF was higher ($P < 0.05$) than in fish fed H3, but neither LF- nor H3-fed fish differed ($P > 0.05$) in 20:5n-3 levels from those fed N6, N3, and N6+3. Levels of 22:5n-3 were not significantly different among fish fed LF, N3, N6+3, and MFO. There was no 22:5n-3 detected in fish fed N6 and H3. The level of DHA (22:6n-3) in fish fed MFO and H3 did not differ significantly and both MFO- and H3-fed fish contained higher ($P < 0.05$) levels of 22:6n-3 than fish fed the other diets. DHA was higher ($P < 0.05$) in fish fed N6+3 than in those fed LF; neither N6+3- nor LF-fed fish differed ($P > 0.05$) in DHA content from those fed N6 and N3.

Liver nonpolar lipid: Total HUFAs in liver nonpolar lipid of fish fed MFO increased significantly by the end of the feeding trial (Figure 5). There was no significant change in HUFA content of fish fed the LF and FAEE diets. In week four, total HUFAs ranged from 1.4 to 29.6% (Table 12). HUFA concentration was higher ($P < 0.05$) in fish fed MFO than in those fed LF, N6, N3, and N6+3, but did not differ ($P > 0.05$) from the HUFA concentration of fish fed H3. Fish fed H3 had higher ($P < 0.05$) HUFA levels than fish fed LF and N6+3, but did not differ ($P > 0.05$) from those fed N6 and N3. Fish fed LF, N6, N3, and N6+3 also did not differ ($P > 0.05$) in HUFA content. EPA (20:5n-3) was not detected in fish fed N6+3, but was present in fish in all other treatment groups. EPA concentrations were not

significantly different among those treatment groups. The level of 22:5n-3 was highest in fish fed MFO. Levels in fish fed N6, N3, and H3 were significantly lower than in fish fed MFO, but did not differ significantly among themselves; 22:5n-3 was not detected in fish fed LF and N6+3. The level of DHA (22:6n-3) in fish fed H3 was higher ($P < 0.05$) than in those fed LF, N6, and N6+3, but did not differ ($P > 0.05$) from 22:6n-3 levels in fish fed N3 and MFO. DHA levels in fish fed LF, N6, N3, N6+3, and MFO were also not significantly different.

In week nine, total HUFAs ranged from 0.2 to 18.5% (Table 13). HUFA levels were higher ($P < 0.05$) in fish fed H3 and MFO than in those fed the other diets. HUFA concentration in fish fed N6+3 was greater ($P < 0.05$) than in fish fed LF, but did not differ ($P > 0.05$ from concentrations in fish fed N6 and N3. Fish fed LF, N6, and N3 did not differ ($P > 0.05$) in HUFA content. EPA (20:5n-3) was higher ($P < 0.05$) in fish fed MFO than in those fed N6, N3, N6+3, and H3, none of which differed ($P < 0.05$) in EPA concentration. EPA was not detected in fish fed LF. The level of 22:5n-3 was higher ($P < 0.05$) in fish fed MFO than in those fed N3 and H3. Fish fed N3 and H3 did not differ significantly in 22:5n-3 concentration; 22:5n-3 was not detected in fish fed LF, N6, and N6+3. DHA (22:6n-3) level was highest ($P > 0.05$) in fish fed H3. There was no significant difference in 22:6n-3 concentrations among fish fed the other diets.

DISCUSSION

Incorporation of purified fatty acid ethyl esters into purified diets represents an artificial situation that would not be applied to real aquaculture practice. However, the use of such diets has its value in that it forces fish to utilize concentrations and combinations of fatty acids to which they would not normally be exposed. Such diets are potentially useful in rapidly eliciting measurable responses in growth and tissue fatty-acid pattern.

Growth (weight gain) of red drum fed FAEE diets was relatively poor. Stickney et al. (1983) reported the poor growth of channel catfish fed diets containing purified fatty acids and suggested growth was affected by the unusual balance of fatty acids in the diets. Satoh et al. (1989a) suggested that reduced growth of fish fed purified diets was due to the poor palatability of such diets. Gatlin et al. (1991) also reported limited acceptability of purified diets by red drum and formulated a semipurified test diet that incorporated adult red drum muscle, which was accepted by the fish. Moon and Gatlin (1994) reported that red drum muscle in the diet supported superior growth of red drum.

In the present study, fish consumed all the diets at about the same rate during the first couple of days. However, the consumption rate of fish on the FAEE diets started to decrease toward the end of the first week of the feeding trial. A decrease in consumption gradually occurred among fish fed the LF diet around the fourth week of

the feeding trial. Diet consumption did not decrease among fish fed MFO, suggesting that diet consumption (palatability) was a factor affecting growth of fish fed the LF and FAEE diets, as well as fatty acid composition.

Fish fed LF had greater weight gain than those fed FAEEs, but inferior weight gain to those fed MFO. Red drum fed MFO had the highest weight gain and feed efficiency among treatment groups. Fish oil is rich in long chain n-3 fatty acids, which are very effective in promoting growth of red drum (Williams and Robinson 1988, Lochmann and Gatlin 1993a, 1993b), channel catfish (Santha & Gatlin 1991), hybrid striped bass (Nematipour and Gatlin 1993a, 1993b), and a variety of other fish species (Watanabe 1982).

Physical EFA Deficiency Sign

"Shock syndrome" (caused by physical irritation of the fish), which is a sign of EFA deficiency in rainbow trout (Sinnhuber 1969), was not observed in this study. Lochmann and Gatlin (1993a, 1993b) reported shock syndrome as a sign of EFA deficiency in red drum of mean initial weight 0.92g, but not in fish of mean initial weight 4.35g. They reasoned that because the initial weights of the fish were larger, they did not incur as severe a degree of EFA deficiency. The relatively large (8-9g) size of red drum used in this study might have precluded observance of shock syndrome as an EFA deficiency sign. However, EFA deficiencies were indicated by the presence of hemorrhages on the body, head, and fins of some fish fed the FAEE diets, and some fed the LF diet. Polar lipids (phospholipids) serve primarily as structural elements of cell membranes. The physical properties of the membrane are determined by its

phospholipid composition. Permeability properties of lipid bilayers in cell membranes are markedly dependent on the nature and chain length of the fatty acid constituents of the phospholipids (Chen et al. 1971, Bell et al. 1986). Under conditions of EFA deficiency, the impermeability of cell membranes in the fish might have been impaired, resulting in hemorrhage. Lochmann and Gatlin (1993a) observed a similar response in red drum fed EFA-deficient diets.

Fatty Acid Composition

Fatty acid composition of fish tissues generally reflected that of the dietary lipid. This relationship has been observed in a variety of fishes, including red drum (Lochmann and Gatlin 1993a, 1993b), hybrid striped bass (Nematipour and Gatlin 1992a, 1992b), rainbow trout (Henderson and Sargent 1981), Atlantic salmon (*Salmo salar*) (Thomassen and Rosjo 1989), grey mullet (*Mugil cephalus*) (Argyropoulou et al. 1992), and white sturgeon (Xu et al. 1993).

Saturated fatty acids (SFAs)

SFAs can be synthesized *de novo* in the liver of animals. In mammals, fatty acid synthetases produce free palmitic acid (16:0) and lesser amounts of stearic (18:0) and myristic acids (14:0) (Sargent et al. 1989). The saturated fatty acids found in the tissue lipids of red drum in this study followed this trend: palmitic acid (16:0) predominated, followed by stearic acid (18:0) and myristic acid (14:0). The bagre manchado (*Pimelodus maculatus*), a freshwater fish in Rio de la Plata, Argentina, produces a high level of palmitic acid when fed a fat-free diet (Brenner et al. 1963). Plaice (*Pleuronectes platessa*) synthesize 16:0 and 18:0 fatty acids in the ratio of 3:2

(Wilson and Williamson 1970). Hansen and Knudsen (1981) reported that over 60% of the radioactivity incorporated from [^{14}C] acetyl-CoA by purified flounder-liver fatty acid synthetase was recovered as 16:0; 15-20% as 18:0; 12-17% as butyric acid (4:0); and only a trace as 14:0. Warman and Bottino (1978) found that catfish liver fatty acid synthetase produced more 18:0 than 16:0 from [^{14}C] acetate *in vitro*. Lytle and Lytle (1994) reported 16:0 to be predominant in the saturated fatty acids in spotted and striped mullet. These findings demonstrated that fatty acid synthesis in fish liver follows a pattern similar to that in mammals, although the saturated products may differ in chain length and fatty acid quantity.

Total SFA levels in tissue lipids of red drum were uniform, regardless of variation in the SFA content of dietary lipid. Similar results have been reported for red drum (Lochmann and Gatlin, 1993a, 1993b), channel catfish (Stickney and Andrews, 1971), rainbow trout (Yu and Sinnhuber, 1972; Yu et al., 1977; Castledine and Buckley, 1980; Greene and Selivonchick, 1990), chinook salmon (*Oncorhynchus tshawytscha*) (Mugrditchian et al., 1981), milkfish (*Chanos chanos* Forskal) (Baustista et al., 1991), and mullet (Argyropoulou et al., 1992; Lytle and Lytle 1994). The similarity in saturated fatty acid composition of tissue lipids, irrespective of dietary treatment, indicates that a mechanism may exist in fish to regulate and maintain saturated fatty acids within narrowly defined physiological levels.

Despite the minimal effect of dietary lipids on tissue SFA concentrations, significant differences in SFA levels did occur over time in some treatment groups. While levels of SFAs in muscle and liver polar lipid of fish fed LF and FAEEs

decreased significantly by the end of the feeding trial, there was no significant change in SFA levels in muscle nonpolar lipid, regardless of treatment, nor were there changes in SFA concentrations in lipid from fish fed MFO (Figure A-2, in appendix, a summary of SFAs in tissue lipids of red drum fed six experimental diets, from information provided in Figures 2-5 and Tables 6-13). Lochmann and Gatlin (1993b) reported an elevation of SFAs in red drum fed a diet with excess (2.5%) n-3 HUFAs. They reasoned that there might be a mechanism to regulate fatty acids in the tissue, by chain-shortening the excess dietary n-3 HUFA *via* peroxisomal β-oxidation, to reduce the excess n-3 HUFA in the body which might be undesirable to the fish. In the present study, fish fed MFO, which contained higher n-3 HUFAs than the other diets had no decrease in SFAs in tissue lipid, while fish fed the LF and FAEE diets did, indicating that little chain-shortening of dietary n-3 HUFA via peroxisomal β-oxidation might have taken place in fish fed MFO.

Fish fed LF had total SFA levels as high or higher than those in tissue lipids of fish fed FAEEs. The LF diet contained very little (~0.15%) lipid, yet the total percentage of body lipid in fish fed LF was not reduced compared to that in fish fed the FAEE diets. It can be assumed that the fatty acids present at low levels in tissue lipids were primarily residual FAs that were present at the initiation of the growth trial, whereas FAs present at high concentrations were a combination of residual FAs and newly synthesized fatty acids. Endogenous fatty acid synthesis in mammals is depressed by increasing levels of dietary lipid and stimulated by increasing levels of dietary carbohydrate respectively (Volpe and Vagelos 1976). Lin et al. (1977) reported

a depressed level of NADPH-producing enzymes (the enzymes that generate acetyl-CoA for fatty acid synthesis in the cytosol) in liver of coho salmon when lipid level in the diet was increased from 5% to 20% at the expense of carbohydrate (dextrin). It is possible that, in this study, fatty acid synthetase in fish fed LF was more active than in fish fed the other diets, because of the low fat content and relatively higher carbohydrate (dextrin) content of the diet. Increased SFA concentration in body lipid of carp fed a low-fat diet (Farkas et al. 1978), and grey mullet fed a fat-free diet (Argyropoulou et al. 1992) has also been reported.

Monoenoic fatty acids (MFAs)

Levels of MFAs in tissue lipids of red drum fed LF and FAEE diets were significantly higher than in those fed MFO (Figure A-3, in appendix, a summary of MFAs in tissue lipids of red drum fed six experimental diets, from information provided in Figures 2-5 and Tables 6-13). The MFA fraction in fish fed FAEEs was primarily oleic acid (18:1n-9) which reflected the high level of 18:1n-9 in the diets. The MFA fraction in fish fed LF was also primarily 18:1n-9, but these fish also contained an appreciable amount of 16:1n-7. High levels of 18:1n-9 and 16:1n-7 may have been due in part to desaturation of 18:0 and 16:0.

Lochmann and Gatlin (1993a, 1993b) also reported an increase in MFAs in red drum fed EFA-deficient diets. Other studies have shown that a lack of dietary essential fatty acids increases tissue levels of monoenoic fatty acids in phospholipid. This has been observed in rainbow trout (Castell et al. 1972c; Takeuchi and Watanabe 1976), carp (*Cyprinus carpio*) (Farkas et al. 1977), chum salmon (*Oncorhynchus keta*)

(Takeuchi et al. 1979), eel (*Anguilla japopnica*) (Takeuchi et al. 1980), milkfish (Baustista and De la Cruz 1988; Borlongan 1992), striped jack (*Longirostris delicatissimus*) (Watanabe et al., 1989), channel catfish (Satoh et al,. 1989b) and red sea bream (Takeuchi et al. 1990). Brenner et al. (1981) stated that elevation of MFA levels in EFA-defficient fish occurs as a result of increased activity of the enzymes involved in monoenoic fatty acid synthesis as a result of EFA deficiency.

Polyunsaturated fatty acids (PUFAs)

PUFA patterns in tissue lipid seemed to be influenced more by dietary 18:3n-3 than 18:2n-6. When 18:3n-3 was present in the diet (N3 and N6+3), n-3 PUFAs became more abundant in tissues of fish fed those diets (Figure A-4, in appendix, a summary of n-3 PUFAs in tissue lipids of red drum fed six experimental diets, from information provided in Figures 2-5 and Tables 6-13). Lochmann and Gatlin (1993b) reported that 18:3n-3 was preferentially metabolized in red drum fed a diet containing equal amounts of linoleate and linolenate due to a competitive inhibition between 18:2n-6 and 18:3n-3 for the Δ-6 desaturase, which preferentially catalyzes the desaturation of n-3 fatty acids. Sellner and Hazel (1982) demonstrated that hepatocytes from rainbow trout had a substrate preference in the order of: 18:3n-3 > 18:2n-6 > 18:1n-9. Yang and Dick (1994) reported that in Arctic Charr (*Salvelinus alpinus*), 18:2n-6 competes with 18:3n-3 for Δ-6, Δ-5 and Δ-4 desaturases, and that 18:3n-3 is preferred over 18:2n-6 for elongation and desaturation. However, in the present study, the increased concentration of n-3 PUFAs in polar lipid of fish fed N3 and N6+3 compared to those fed the other diets consisted primarily of 18:3n-3, which originated from the diets.

Derivatives of 18:3n-3 were small in amount and no greater in concentration than those of 18:2n-6. Evidence of preferential specificity of desaturases was not apparent in the PUFA composition of red drum in this study.

Interestingly, unlike n-3 PUFAs, the level of n-6 PUFAs generally decreased during the feeding trial, even in fish fed diets supplemented with 18:2n-6 (except in muscle polar lipid of fish fed LF, N6, and N6+3; in muscle nonpolar lipid of fish fed all diets) (Figure A-5, in appendix, a summary of n-6 PUFAs in tissue lipids of red drum fed six experimental diets, from information provided in Figures 2-5 and Tables 6-13). The way that n-6 PUFAs were utilized in red drum is not clear. However, concentrations of n-6 PUFAs in liver lipids (polar and nonpolar) of fish were much lower than concentrations of n-6 PUFAs in muscle lipids, regardless of diet fed, indicating that n-6 PUFAs might have been utilized for energy. Despite the overall decrease in n-6 PUFA levels during the feeding trial, arachidonic acid (20:4n-6) was conserved in muscle polar lipid of all fish in similar concentrations (though lower in fish fed LF). Mead (1968) indicated that preservation of essential fatty acids occurs only after incorporation into certain phospholipids, particularly if phospholipid becomes part of a membrane or other more permanent structure. The preservation of 20:4n-6 in muscle polar lipid of red drum suggested a specific functional role for this fatty acid in the fish. Arachidonic acid (20:4n-6) is the major PUFA in phosphatidylinositol (PI) from marine fish (Bell et al. 1983, Bell et al. 1985). PI is metabolically active, playing a pivotal role in the transduction of hormone signals through biomembranes. It is believed that 20:4n-6 is an essential fatty acid in marine fish (Bell et al. 1986).

Some derivatives of 18:2n-6 and 18:3n-3 such as γ-linolenic acid (18:3n-6), eicosadienoic acid (20:2n-6), di-homo-γ-linolenic acid (20:3n-6) and 18:4n-3, that were not detected initially in the fish, were detected in the polar fraction of muscle and lipid (more often in the fish fed diets containing either 18:2n-6 and/or 18:3n-3), indicating Δ-6 desaturase activity. Although these fatty acids were found in very low concentrations, their presence indicates that red drum have a limited ability to elongate and desaturate 18:2n-6 and 18:3n-3 to longer-chain, more-unsaturated forms.

Highly unsaturated fatty acids (HUFAs)

The pattern of highly unsaturated fatty acids in tissue lipid was most strongly influenced by dietary HUFA content. Fish fed diets that did not contain HUFAs, exhibited depleted levels of HUFAs in the polar fraction of tissue lipid (Figure A-6, in appendix, a summary of HUFAs in tissue lipids of red drum fed six experimental diets, from information provided in Figures 2-5 and Tables 6-13), which indicated that HUFAs were either not synthesized or were produced too slowly to supply tissue needs. Fish fed the diet containing 22:6n-3 ethyl ester (H3) had no significant change in total HUFA concentration in tissue lipid, while fish fed MFO significantly increased in total HUFA concentration in tissue lipid. Fish fed diets containing 18:3n-3 (N3 and N6+3) did not have HUFA levels any higher than fish fed diets that did not contain 18:3n-3 (LF and N6), confirming that the red drum has a limited ability to chain-elongate and desaturate fatty acids. Similar results have been reported by Lochmann and Gatlin (1993a, 1993b).

N-3 HUFAs were conserved in the polar fraction of tissue lipid in all fish, regardless of diet fed, indicating the importance of highly unsaturated fatty acids as components of phospholipids. HUFAs probably serve no special function in depot lipid (nonpolar lipid), since HUFA concentrations in nonpolar fractions of tissue lipid were much lower compared to those in the polar fraction of tissue lipid. However, there were differences in the way 20:5n-3 and 22:6n-3 were conserved in tissue lipid.

In muscle and liver lipids of fish in all treatment groups, 22:6n-3 was present in higher concentration than 20:5n-3, even in fish fed the MFO diet that was higher in 20:5n-3 concentration than 22:6n-3 (except in nonpolar lipid of fish fed MFO, which had higher 20:5n-3 than 22:6n-3 levels). This selective retention indicates that 22:6n-3 may be more important, or is required in higher amount, than 20:5n-3 for membrane structure and normal function of fish.

EFA activity of HUFAs: Studies to determine the essential fatty acid (EFA) requirements of fish, using purified diets, appear to support a classification of requirements based on the effect of water temperature as well as the effect of natural feeding habits on fish biochemical functions (Cowey and Sargent 1979). Marine carnivores, like red sea bream and turbot, require highly unsaturated n-3 fatty acids-eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) for optimum growth (Cowey et al. 1976, Yone 1978). *Tilapia zillii*, a euryhaline, tropical, herbivorous fish grows best when fed diets containing 18:2n-6 and 20:4n-6 rather than 18:3n-3 and 20:5n-3 (Kanazawa et al. 1980). Rainbow trout, a freshwater, coldwater carnivore can utilize both 18:3n-3 and the more highly unsaturated fatty acids in the n-3

series as EFA sources (Castell et al. 1972b, Takeuchi and Watanabe 1982). Warmwater fishes living in freshwater seem to require both n-6 and n-3 unsaturated fatty acids (Stickney and Hardy 1989). Watanabe (1993) reported that in marine larval fish, DHA is superior in EFA activity to EPA. He suggested that there is a functional difference between EPA and DHA, and unbalanced ratios of the two can lead to high mortality in larval fish. In this study, absolute EFA requirements for red drum were not determined, but the MFO diet, which was rich in n-3 HUFA, supported the best growth. It is assumed from this observation that the red drum, like most marine fishes, has an EFA requirement for n-3 HUFA (EPA and DHA). Similar results for red drum fed purified fatty acids were reported by Lochmann and Gatlin (1993a, 1993b). Webster et al. (1994) reported that fasted channel catfish conserved DHA in muscle and liver tissues, implying that channel catfish had a metabolic or structural need for DHA. It remains to be determined whether EPA or DHA is more important for red drum and the appropriate dietary level of each.

Bell et al. (1986) observed that there is no physio-chemical basis for assuming that differences exists in the abilities of 20:5n-3, 20:4n-6 and 22:6n-3 to confer fluidity to cellular membranes, because all HUFAs are fluid at temperatures considerably below those found in biological systems. Lee et al. (1986) suggested that the essential nature of a fatty acid is related to some property other than its role in membrane fluidity. Red drum may lack the necessary elongation and desaturation enzymes required to convert PUFAs to HUFAs and might have an absolute requirement for all three long-chain highly unsaturated fatty acids (20:4n-6, 20:5n-3, and 22:6n-3).

HUFA ratios as EFA deficiency indicators: In some animals and fish, a fat-free diet or an EFA-deficient diet results in an elevated level of eicosatrienoic acid (20:3n-9) in phospholipids. This occurs in the rat (Mohrhauer and Holman 1963), chick (Hill 1966), and rabbit (Ahluwalia et al. 1967). Since most animals require EFA of the linoleic family, Mohrhauer and Holman (1963) suggested the ratio of 20:3n-9/20:4n-6 in lipid as an index of EFA deficiency. A ratio of 0.4 or greater indicates EFA deficiency in mammals (Watanabe 1982). Because the rainbow trout has an EFA requirement for linolenate-family fatty acids, the ratio of 20:3n-9 to 22:6n-3 has been proposed as an index of EFA adequacy for rainbow trout (Castell et al. 1972b). Carp require both 18:2n-6 and 18:3n-3. Watanabe et al. (1975) proposed both 20:3n-9/20:4n-6 ratio and 20:3n-9/22:6n-3 ratio as EFA indices for carp. Satoh et al. (1989a) reported that channel catfish fed equal amounts of 18:2n-6 and 18:3n-3 had a 20:3n-9/22:6n-3 ratio of 0.5, but exhibited a poor growth rate. They suggested that 20:3n-9/22:6n-3 ratio is not a suitable ratio for determination of EFA deficiency in channel catfish. Takeuchi et al. (1980) also reported that 20:3n-9/22:6n-3 ratio is not a good index for EFA status in the eel.

Increased levels of 20:3n-9 were not detected in red drum in this study, nor have they been observed in other studies with red drum (Lochmann and Gatlin 1993a, 1993b), plaice and turbot (Owen et al. 1975), and sunshine bass (*Morone chrysops* F x *M. saxatilis* M) (Nematipour and Gatlin 1993a), due to the limited ability of these fish to produce 20:3n-9 from shorter-chain monoenoic fatty acids. Therefore, the ratio of 20:3n-9/(n-3 HUFA) does not appear to be an appropriate index for EFA deficiency in

red drum and other marine fishes. Watanabe (1982) suggested it is impossible to decide from EFA index alone whether or not fish are receiving a sufficient amount of EFA to maximize growth. Evaluation of EFA status should be done from physiological, histological, and biochemical perspectives.

Factors Affecting Tissue Fatty Acid Composition

Water temperature determines the degree of unsaturation of polyunsaturated fatty acids (PUFA) in membrane phospholipids required to maintain membrane fluidity (Bell et al. 1986). In that respect, n-3 PUFAs can offer a higher degree of unsaturation than n-6 PUFAs. Experiments with eel have shown that the ratio of n-3/n-6 fatty acids in gill tissue increased with a decrease in temperature (Thomson et al. 1975). Similarly a decrease in temperature resulted in the increase of PUFA and especially n-3 fatty acids in phospholipids of trout liver (Hazel 1979).

The aquatic environment (freshwater or seawater) and natural food preferences (herbivorous-carnivorous) determine the main type of fatty acids consumed under natural conditions and also the capacity of fish to further elongate and desaturate them (Cowey and Sargent 1979). Freshwater fish consume a diet that is partly terrestrial in origin. The lipid in this diet is rich in n-6 fatty acids and short-chain n-3 PUFA. Marine fish and especially marine carnivores have a natural diet rich in highly unsaturated n-3 fatty acids. As a consequence, levels of n-6 fatty acids and short chain-n-3 PUFAs are higher in tissues of freshwater fish than marine fish, the latter having higher concentrations of long-chain n-3 PUFA (Ackman 1967). In addition to diet intake, environmental factors (temperature and salinity) also affect the tissue fatty acid

composition of fish. Furthermore, temperature and salinity of the water also play a role in the capacity of fish to chain-elongate and desaturate fatty acids. Ability to chain-elongate and desaturate has a great impact on requirements for essential fatty acids, because fish that are capable of producing long-chain PUFAs are better able to maintain proper fatty acid composition of membrane functions in their "optimal" state. Such adaptation is vital for fish survival, especially in an environment with large temperature fluctuations, and may explain in part the differences in tissue fatty acid composition and the efficiency of fatty acid chain elongation and desaturation between freshwater and marine fishes.

Freshwater fish live in relatively shallow waters that may exhibit considerable diurnal and seasonal variation. To survive in this kind of environment, fish require a means to efficiently elongate and desaturate fatty acids to maintain cell membrane fluidity. Marine fish live in deep, large water bodies where water temperature is comparatively lower, but diurnal fluctuation of temperatures is not as pronounced. Thus, for marine fish, the ability to chain elongate and desaturate fatty acids to adjust membrane fluidity may not be as great as in freshwater fish. However, many marine fish, which spend much of their lives at low water temperatures, require longer chain polyunsaturated fatty acids of the (n-3) series and/or the (n-6) series as dietary essential fatty acids to keep a certain level of membrane fluidity at all times.

Implications for the Red Drum Aquaculture Industry

One of the drawbacks to culturing red drum in Louisiana is the fish's intolerance of cold temperature. The cold front that entered Louisiana in December 1989 killed

much of that year's red drum crop and discouraged many potential redfish farmers (Petrocci 1993). HUFAs are essential for the survival of fish in low temperatures. However, farm-raised red drum has lower HUFA content than wild red drum (Jahncke et al. 1988., Nettleton 1990, and Villarreal et al. 1994), due to the fact that most commercial fish feed being used today provides the fish with lower amount of HUFAs than does their natural diet in the wild. In addition, red drum, like other marine fishes, are not as efficient as freshwater fish in chain-elongating and desaturating fatty acids, thus making the fish incapable of adjusting membrane fluidity rapidly enough to adapt to sudden changes in temperature.

As studies have shown, dietary intake has a major effect on tissue fatty acid composition. Selection of feeds with proper fatty acid composition must have some bearing on adaptation of fishes to their environment. The actual level of eicosapentaenoic acid and docosahexenoic acid in fish tissue can be manipulated by dietary means (Farkas 1980). It is possible that a feed could be formulated for red drum to increase the n-3 HUFA content in tissue so as to enable the fish to survive cold temperatures. This has not yet been attempted with any fish species. Further studies are necessary to determine the appropriate amount of HUFA for adaptation of red drum to decreased temperature. If the idea of a "winter feed" is feasible, it could be of economic significance and contribute to the success of the red drum aquaculture industry.

Benefits for Human Nutrition

Besides the "low fat, low calorie, high protein" image of fish, fish is also known for its high n-3 HUFA content which is not readily available in other protein sources for human consumption. The HUFAs that confer the greatest health benefits to humans are EPA and DHA. There is convincing evidence that consumption of n-3 HUFA in fish oil reduces the risk of heart disease. The low incidence of atherosclerotic disease among Greenland Eskimos was attributed in part to their high fish consumption (Dyerberg et al. 1978). However, wild-caught fish and farm-raised fish are different in fat content, mainly due to differences in diets. Farmed-raised fish fed grain-based diets have a fatty acid composition that is much lower in n-3 PUFA than wild-caught fish (Jahncke et al. 1988, Nettleton 1990). Due to growing health concerns associated with dietary fat intake in humans, researchers are pursuing the possibility of altering the quality of lipid in fish by manipulating lipid in fish feeds. Since the fatty acid content of farm-raised fish is highly dependent upon the diet, it is possible to increase the n-3 fatty acid content of fish by changing the amount and kind of oil in fish feed. Studies have demonstrated this in white sturgeon (Xu et al. 1993) and hybrid striped bass (Fowler et al. 1994).

Marine fish oils are more costly than other lipid sources. To reduce feed cost, a "finishing diet" rich in n-3 HUFAs could be formulated for use about four weeks prior to harvest. As results of the present study showed, significant changes in tissue fatty acid composition occurred after four weeks of feeding. However, nutritional properties of fish are not the only attributes that concern consumers; sensory properties

must also be considered. HUFAs are susceptible to oxidation and their oxidation products are known contributors to fishy flavor and aroma. Fowler et al. (1994) reported that concentrations of EPA and DHA in edible muscle tissue of hybrid striped bass directly affected fishy-flavor intensities. They suggested that antioxidants such as alpha-tocopherols in the fish muscle may help to prevent oxidation reactions and help minimize formation of fish flavors.

In the earlier days of aquaculture, ingredients in diets were determined largely by cost rather than by their contribution to the nutritive value of the fish to the consumer. As the volume of cultured fish increases and markets expand, more emphasis may be placed on the nutritional value of fish products. Pigott (1989) stated that now is the time for the aquaculture industry to alter fish feeding programs to ensure that HUFA n-3 fatty acids are available in fish products. The idea of enhancing the nutrient profile of an already excellent food through changes in fat content and composition is a possibility at the forefront of both aquaculture and nutrition. It is an opportunity that demands full support from the concerned organizations for the necessary research.

CONCLUSIONS

Fish fed the low-fat diet grew better than those fed FAEEs, but inferior to those fed menhaden fish oil. Fish fed MFO had the best growth response. The effects of dietary lipids were more apparent in the polar than in the nonpolar fraction of tissue lipids. Levels of saturated fatty acids in tissue lipids were narrowly regulated despite dietary treatment. However, fish fed LF and FAEE diets had a decreased level of total SFAs in tissue, while fish fed MFO did not. Levels of monoenoic fatty acids were elevated in fish fed LF and FAEE but reduced in fish fed MFO. N-6 PUFAs were depleted in fish fed diets that did not contain 18:2n-6. Arachidonic acid (20:4n-6) was spared in muscle polar lipid, indicating a functional role for 20:4n-6 in the fish. N-3 PUFAs increased in muscle lipid of fish fed diets containing 18:3n-3. N-3 HUFAs were depleted in polar lipid of fish fed diets devoid of n-3 HUFAs. N-3 HUFAs were conserved in polar lipid in higher concentration of DHA than that of EPA. Red drum showed a limited ability to chain-elongate and desaturate fatty acids. The data obtained in this experiment indicate that the red drum appears to have a dietary requirement for 20:5n-3, 22:6n-3, and 20:4n-6. The fatty acid composition of muscle and liver tissues of red drum generally reflected the dietary fatty acid pattern. This suggests that by feed manipulation, n-3 HUFAs in red drum could be enhanced to increase the nutritive value of red drum for human consumption. N-3 HUFA enhancement might also improve low-temperaure tolerance of pond-raised fish.

REFERENCES

Ackman, R.G. 1967. Characteristics of the fatty acid composition and biochemistry of some freshwater fish oils and lipids in comparison with marine oils and lipids. Comparative Biochemistry and Physiology 22: 907-922.

Ahluwalia, B.G., G. Pincus, and R.T. Holman. 1967. Essential fatty acid deficiency and its effect upon reproductive organs of male rabbits. Journal of Nutrition 92: 205-214.

Allen, W.V. 1976. Biochemical aspects of lipid storage and utilization in animals. American Zoology 16: 31-647.

Andrews, J.W., M.W. Murray, and J.M. Davis. 1978. The influence of dietary fat levels and environmental temperature on digestible energy and absorbability of animal fat in catfish diets. Journal of Nutrition 108: 749-752.

Argyropoulou, V., N. Kalogeropoulos, and M.N. Alexis. 1992. Effect of dietary lipids on growth and tissue fatty acid composition of grey mullet (*Mugil cephalus*). Comparative Biochemistry and Physiology 101A(1): 129-135.

Arnold, C.R. 1988. Controlled year-round spawning of the red drum (*Sciaenops occellatus*) in captivity. Contributions in Marine Science 30 (Supplement): 65-70.

Bass, R.J., and J.W. Avault, Jr. 1975. Food habits, length-weight relationship, condition factor, and growth of juvenile red drum, *Sciaenops ocellatus*, in Louisiana. Transaction of American Fisheries Society 1: 35-46.

Bautista, M.N., and De la Cruz, M.C. 1988. Linoleic (n-6) and linolenic (n-3) acids in the diet of fingerling milkfish (*Chanos chanos* Forskal). Aquaculture 71: 347-358.

Bautista, M.N., M.J. del Valle, and F.M. Orejana. 1991. Lipid and fatty acid composition of brackishwater- and freshwater-reared milkfish (*Chanos chanos* Forskal). Aquaculture 96: 241-248.

Bell, M.V., C.M.F. Simpson, and J.R. Sargent. 1983. (n-3) and (n-6) polyunsaturated fatty acids in the phospholglycerides of salt-secreting epithelia from two marine fish species. Lipids 18: 720-726.

Bell, M.V., R.J. Henderson, and J.R. Sargent. 1985. Changes in the fatty acid composition of phospholipids from turbot (*Scophthalmus maximus*) in relation to dietary polyunsaturated fatty acid deficiencies. Comparative Biochemistry and Physiology 81B: 193-198.

Bell, M.V., R.J. Henderson, and J.R. Sargent. 1986. The role of polyunsaturated fatty acids. Comparative Biochemistry and Physiology 83B(4): 711-719.

Boothby, R.N. and J.W. Avault, Jr. 1971. Food habits, length-weight relationship, condition factor, and growth of juvenile red drum, *Sciaenops ocellata*, in Southeastern Louisiana. Transaction of American Fisheries Society 2: 290-295.

Borlongan, I.G. 1992. The essential fatty acid requirement of milkfish (*Chanos chanos* Forsskal). Fish Physiology and Biochemistry 9: 401-407.

Borlongan, I.G., and L.V. Benitez. 1992. Lipid and fatty acid composition of milkfish (*Chanos chanos* Forsskal) grown in freshwater and seawater. Aquaculture 104: 79-89.

Brenner, R.R. 1981. Nutritional and hormonal factors influencing desaturation of essential fatty acids. Progress in Lipid Research 20:41-47.

Brenner, R.R., D.V. Vazza, and M.E. De Tomas. 1963. Effect of a fat-free diet and of different dietary fatty acids (palmitate, oleate, and linoleate) on the fatty acid composition of fresh-water fish lipids. Journal of Lipid Research 4(3): 341-345.

Brown, P.B., D.A. Davis, and E.H. Robinson. 1988. An estimate of the dietary lysine requirement of juvenile red drum *Sciaenops ocellatus*. Journal of the World Aquaculture Society 19(3): 109-112.

Castell, J.D., R.O. Sinnhuber, D.J. Lee, and J.H. Wales. 1972a. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): physiological symptoms of EFA deficiency. Journal of Nutrition 102: 87-92.

Castell, J.D., D.J. Lee, and R.O. Sinnhuber. 1972b. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): lipid metabolism and fatty acid composition. Journal of Nutrition 102: 93-100.

Castell, J.D., D.J. Lee, J.H. Wales, and R.O. Sinnhuber. 1972c. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): growth, feed conversion and some gross deficiency symptoms. Journal of Nutrition 102:77-86.

Castledine, A.J., and J.T. Buckley. 1980. Distribution and mobility of n-3 fatty acids in rainbow trout fed varying levels and types of dietary lipid. Journal of nutrition 110: 675-685.

Chen, L.F., D.B. Lund, and T. Richardson. 1971. Essential fatty acids and glucose permeability of lecithin membranes. Biochimica Biophysica Acta 225: 89-95.

Cowey, C.B., J.M. Owen, J.W. Adron, and C. Middleton. 1976. Studies on the nutrition of marine flatfish. The effect of different dietary fatty acids on the growth and fatty acid composition of turbot (*Scophthalmus maximus*). British Journal of Nutrition 36: 479-486.

Cowey, C.B., and L.R. Sargent. 1979. Nutrition. *In* Fish Physiology. Edited by Hoar, W.S., D.J. Randall, and J.R. Brett. Vol III. pp 1-69. Academic Press. Orlando.

Craig, S.R., and D.M. Gatlin III. 1992. Dietary lysine requirement of juvenile red drum *Sciaenops ocellatus*. Journal of the World Aquaculture Society 23(2): 133-137.

Daniels, W.H., and E.H. Robinson. 1986. Protein and energy requirements of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 53: 243-252.

Davis, D.A., and E.H. Robinson. 1987. Dietary phosphorus requirement of juvenile red drum, *Sciaenops ocellatus*. Journal of World Aquaculture Society 18: 129-136.

Dyerberg, J., H.O. Bang, E. Stoffersen, S. Moncada, and J.R. Vane. 1978. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. Lancet July(2): 117-119.

Ellis, S.C. and R.C. Reigh. 1991. Effects of dietary lipid and carbohydrate levels on growth and body composition of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 97: 279-292.

Farkas, T., I. Csengeri, F. Majoros, and J. Olah. 1980. Metabolism of fatty acids in fish. III. Combined effect of environmental temperature and diet on formation and deposition of fatty acids in the carp, *Cyprinus carpio* Linnaeus 1758. Aquaculture 20: 29-40.

Farkas, T. I. Csengeri, F. Majoros, and J. Olah. 1977. Metabolism of fatty acids in fish. I. Development of essential fatty acid deficiency in the carp, *Cyprinus carpio* linnaeus 1758. Aquaculture 11: 147-157.

Farkas, T. I. Csengeri, F. Majoros, and J. Olah. 1978. Metabolism of fatty acids in fish.II. Development of essential fatty acid deficiency in the carp, *Cyprinus carpio* linnaeus 1758. Aquaculture 14: 57-65.

Folch, J., M. lees and G.H.S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226: 497-509.

Fowler, K.P., C. Kkarahadien, N.J. Greenberg, and R.M. Harrell. 1994. Composition and quality aquacultured hybrid striped bass fillets as affected by dietary fatty acids. Journal of Food Science 59(1): 70-75.

Gatlin, D.M.,III, J.P. O'Connell, and J. Scarpa. 1991. Dietary zinc requirement of the red drum, *Sciaenops ocellatus*. Aquaculture 92: 259-265.

Gatlin, D.M.,III, D.S. MacKenzie, S.R. Craig, and W.H. Neill. 1992. Effects of dietary sodium chloride on red drum juveniles in waters of various salinities. The Progressive Fish-Culturist 54: 220-227.

Greene, D.H.S., and D.P. Selivonchick. 1987. Lipid metabolism in fish. Progressive Lipid Research 26: 53-85.

Greene, D.H.S., And D.P. Selivonchick. 1990. Effects of dietary vegetable, animal and marine lipids on muscle lipid and hematology of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 89: 165-182.

Hansen, H.O., and J. Knudsen. 1981. The influence of environmental and incubation temperature on fatty acid synthetae from flounder (*Platichthys flesus* L.) liver. Comparative Biochemistry and Physiology 70B: 515-520.

Hazel, J.R. 1979. Influence of thermal acclimation on membrane lipid composition of rainbow trout liver. American Journal of Physiology 236: R91-R101.

Henderson, R.J., and J.R. Sargent. 1981. Lipid biosynthesis in rainbow trout, *Salmo gairdneri*, fed diets of differing in lipid content. Comparative Biochemistry and Physiology 69C: 31-37.

Hill, E.G. 1966. Effect of dietary linoleate on chick liver fatty acids: dietary linoleate requirement. Journal of Nutrition 89: 465-471.

Holt, G.J. 1992. Experimental studies of feeding in larval red drum. Journal of the World Aquaculture Society 23(4): 265-270.

Holt, G.J., and C.R. Arnold. 1983. Effects of ammonia and nitrite on growth and survival of red drum eggs and larvae. Transactions of the American Fisheries Society 112: 314-318.

Holt, G.J., A.G. Johnson, C.R. Arnold, W.A. Fable, Jr., and T.D. Williams. 1981. Description of eggs and larvae of laboratory reared red drum, *Sciaenops ocellata*. Copeia 1981: 751-756.

Holt, G.J. , C.R. Arnold, and C.M. Riley. 1990. Intensive culture of larval and post-larval red drum. Pages 53-56 in G.W. Chamberlain, R.J. Miget and M.g. Haby, editors. Manual on red drum aquaculture. Texas A&M Sea Grant College Program Np. TAMU-SG-90-603, College Station, Texas, USA.

Jahncke, M., J.B. Hale, J.A. Gooch, and J.S. Hopkins. 1988. A research note: Comparison of pond-raised and wild red drum (*Sciaenops ocellatus*) with respect to proximate composition, fatty acid profiles, and sensory evaluations. Journal of Food Science 53(1):286-287.

Jahncke, M. L., T.I.J. Smith, and G.T. Seaborn. 1989. Use of fatty acid profiles to distinguish cultured from wild fish: a possible law enforcement tool. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 42(1988): 546-553.

Kanazawa, A., S. Teshima, M. Sakamoto, and M.A. Awal. 1980. Requirements of *Tilapia zillii* for essential fatty acids. Bulletin of the Japanese Society of Scientific Fisheries 46(11): 1353-1356.

Kayama, M. 1966. Fatty acid metabolism in fish. Japanese Society of Scientific Fisheries 30: 649-659.

Lee, A.G., J.M. East, and R.J. Fround. 1986. Are essential fatty acids essential for membrane function? Progressive Lipid Research 25: 41-46.

Lee, W.Y., G.J. Holt and C.R. Arnold. 1984. Growth of red drum larvae in the laboratory. Transactions of the American Fisheries Society 113: 243-246.

Lin, H., D.R. Romsos, P.I. Tack, and G.A. Leveille. 1977. Effects of fasting and feeding various diets on hepatic lipogenic enzyme activities in coho salmon (*Oncorhynchus kisutch* (Walbaum)). Journal of Nutrition 107: 1477-1483.

Lin, H., and C.R. Arnold. 1983. The growth response of redfish (*Sciaenops ocellatus*) to prepared diets. Annual World Mariculture Society Meeting, Washington, DC. 106 pp.

Lochmann, R.T., and D.M. Gatlin III. 1993a. Essential fatty acid requirement of juvenile red drum (*Sciaenops ocellatus*). Fish Physiology and Biochemistry 12(3): 221-235.

Lochmann, R.T., and D.M. Gatlin III. 1993b. Evaluation of different types and levels of triglycerides, singly and in combination with different levels of n-3 highly unsaturated fatty acid ethyl esters in diets of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 114: 113-130.

Lorio, W., G. Lutz, and J. Avery. 1993. Aquaculture Factsheet - Louisiana Aquaculture as of 1993. Louisiana Cooperative Extension Service. Agricultural Center. Louisiana State University.

Louisiana Aquaculture. 1990. Redfish. Louisiana Cooperative Extension Service. Agricultural Center. Louisiana State University.

Lovell, T. 1989. The nutrients. pp.11-71. *In* Nutrition and feeding of fish. An AVI Book. Published by Van Nostrand Reinhold. New York.

Lytle, J.S., and T. F. Lytle. 1994. Fatty acid pattern differences among individuals of two esturarine fishes (*Leiostomus xanthurus* and *Mugil cephalus*). Gulf Research Reports 9(1): 49-56.

Mead, J.F. 1968. The metabolism of the polyunsaturated fatty acids. In R.T. Holman (ed.), Progress in the chemistry of fats and other lipids, 9: 161-194. Pergamon Press, Oxford.

Mohrhauer, H., and R.T. Holman. 1963. Effect of linolenic acid upon the metabolism of linoleic acid. Journal of Nutrition 81:67-74.

Moon, H.Y., and D.M. Gatlin III. 1991. Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 95:97-106.

Moon, H.Y., and D.M. Gatlin III. 1994. Effects of dietary animal proteins on growth and body composition of the red drum (*Sciaenops ocellatus*). Aquaculture 120:327-340.

Morrison, W.R., and L.M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Researach 5: 600-608.

Mugrditchian, D.S., R.W. Hardy, and W.T. Iwaoka. 1981. Linseed oil and animal fat as alternative lipid sources in dry diets for chinook salmon *Oncorhynchus tshawytscha*. Aquaculture 25: 161-172.

Nematipour, G.R., and D.M. Gatlin III. 1993a. Effects of different kinds of dietary lipid on growth and fatty acid composition of juvenile sunshine bass, *Morone chrysops* (F) x *M. savatilis* (M). Aquaculture 114: 141-154.

Nematipour, G.R., and D.M. Gatlin III. 1993b. Requirement of hybrid striped bass for dietary (n-3) highly unsaturated fatty acids. Journal of Nutrition 123: 744-753.

National Research Council. 1983. Nutrient requirements of warmwater fishes and shellfishes. National Academy Press, Washington, DC.

Nettleton, J.A. 1990. Comparing nutrients in wild and farm fish. Aquaculture Magazine January/February:34-41.

Owen, J.M., J.W. Adron, C. Middleton, and C.B. Cowey. 1975. Elongation and desaturation of dietary fatty acids in turbot *Scophthalmus maximus* L., and rainbow trout, *Salmo gairdnerii*. Lipids 10(9): 528-531.

Petrocci, C. 1993. Redfish round-up: Texas style. Aquaculture Magazine September/October:46-51.

Pigott, G.M. 1989. The need to improve omega-3 content of cultured fish. World Aquaculture 20(1): 63-68.

Pursley, G.G., and W.R. Wolters. 1989. Water quality affects growth of young redfish. Louisiana Agriculture 32: 14-15.

Raven, D. 1994. Redfish. Seafood Learder March/April: 105-112

Reigh, R.C. and S.C. Ellis. 1992. Effects of dietary soybean and fish protein ratios on growth and body composition of red drum (*Sciaenops ocellatus*) fed isonitrogenous diets. Aquaculture 104: 279-292.

Robinson, E.H. 1991. Red drum, *Sciaenops ocellatus*. In CRC Handbook of Nutrient Requirements of Finfish. R.P. Wilson (Editor).

Santha, C.R., and D.M. Gatlin III. 1991. Growth response and fatty acid composition of channel catfish fry fed practical diets supplemented with menhaden fish oil. The progressive Fish-Culturist 53: 135-140.

Sargent, J., R.J. Henderson, and D.R. Tocher. 1989. The lipids. *In* Fish Nutrition. pp.153-218. J.E. Halver (Editor). Academic Press, Inc. New York.

Satoh, S., W.E. Poe, and R.P. Wilson. 1989a. Studies on the essential fatty acid requirement of channel catfish, *Ictalurus punctatus*. Aquaculture 79: 121-128.

Satoh, S., W.E. Poe, and R.P. Wilson. 1989b. Effect of dietary n-3 fatty acids on weight gain and liver polar lipid fatty acid composition of fingerling channel catfish. Journal of nutrition 119: 23-28.

Sellner, P.A., and J.R. Hazel. 1982. Incorporation of polyunsaturated fatty acids into lipids of rainbow trout hepatocytes. American Journal of Physiology 243: R223-R228.

Serrano, J.A., G.R. Nematipour, and D.M. Gatlin III. 1992. Dietary protein requirement of the red drum (*Sciaenops ocellatus*) and relative use of dietary carbohydrate and lipid. Aquaculture 101: 283-291.

Sinnhuber, R.O. 1969. The role of fats. *In* Fish in Research. pp.245-261. O.W. Neuhaus and J.E. Halver (Editors). Academic Press, New York.

Skonberg, D.I., B.A. Rasco, and F.M. Dong. 1994. Fatty acid composition of salmonid muscle changes in response to a high oleic acid diet. Journal of Nutrition 124: 1628-1638.

Statistical Analysis Systems. 1988. SAS/STAT user's guide, release 6.03 edition, SAS Institute, Inc., Cary, North Carolina, USA.

Stickney, R.R., and J.W. Andrews. 1971. Combined effects of dietary lipids and environmental temperature on growth, metabolism and body composition of channel catfish (*Ictalurus punctatus*). Journal of Nutrition 101: 1703-1710.

Stickney, R.R., and J.W. Andrews. 1972. Effects of dietary lipids on growth, food conversion, lipid and fatty acid composition of channel catfish. Journal of Nutrition 102: 249-258.

Stickney, R.R. and R.B. McGeachin. 1983. Effects of dietary lipid quality on growth and food conversion of tilapia. Proceedings. Annual Conference. Southeastern Association of Fish and Wildlife Agencies 37: 352-357.

Stickney, R.R., R.B. McGeachin, D.H. Lewis, and J. Marks. 1983. Response of young channel catfish to diets containing purified fatty acids. Transactions of the American Fisheries Society 112: 665-669.

Stickney, R.R. and R.W. Hardy. 1989. Lipid requirements of some warmwater species. Aquaculture 79: 145-156.

Sukenik, A., and R. Wahnon. 1991. Biochemical quality of marine unicellular algae with special emphasis on lipid composition. I. *Isochrysis galbana*. Aquaculture 97: 61-72.

Takaeuchi, T. T. Watanabe. 1978. Growth-enhancing effect of cuttlefish liver oil and short-necked clam oil on rainbow trout and their effective components. Bulletin of the Japanese Society of Scientific Fisheries 44(7): 733-738.

Takeuchi, T., T. Watanabe. 1977a. Requirement of carp for essential fatty acids. Bulletin of the Japanese of Scientific Fisheries 43(5): 541-551.

Takeuchi, T., and T. Watanabe. 1977b. Effect of eicosapentaenoic acid and docosahexaenoic acid in pollock liver oil on growth and fatty acid composition of rainbow trout. Bulletin of the Japanese of Scientific Fisheries 43(8): 947-953.

Takeuchi, T., S. Arai, T. Watanabe, and Y. Shimma. 1980. Requirement of eel (*Anguilla japonica*) for essential fatty acids. Bulletin of the Japanese Society of Scientific Fisheries 46(3): 345-353.

Takeuchi, T., and T. Watanabe. 1976. Nutritive value of n-3 highly unsaturated fatty acids in pollock liver oil for rainbow trout. Bulletin of the Japanese Society of Scientific Fisheries 42(8): 907-919.

Takeuchi, T., S. Satoh, and T. Watanabe. 1983. Requirement of *Tilapia nilotica* for essential fatty acids. Bulletin of the Japanese Society of Scientific Fisheries 49(7): 1127-1134.

Takeuchi, T., M. Toyota, S. Satoh, and T. Watanabe. 1990. Requirement of juvenile red seabream *Pagrus major* for eicosapentaenoic and docosahexaenoic acids. Bulletin of the Japanese Society of Scientific Fisheries 56: 1263-1269.

Takeuchi, T., and T. Watanabe. 1982. Effects of various polyunsaturated fatty acids on growth and fatty acid compositions of rainbow trout *Salmo gairdneri*, coho salmon *Oncorhynchus kisutch*, and chum salmon *Onchorhynchus keta*. Bulletin of the Japanese Society of Scientific Fisheries 48: 1745-1752.

Thomas, R.G., and W.R. Wolters. 1992. Factors affecting the survival of fingerling red drum in freshwater ponds. The Progressive Fish-Culturist 54: 215-219.

Thomassen, M.S. and C. Rosjo. 1989. Different fats in feed for salmon: influence on sensory parameters, growth rate and fatty acids in muscle and heart. Aquqculture 79: 129-135.

Thomson, A.J., J.R. Sargent and J.M. Owen. 1975. Effect of environmental changes on the lipid composition and (Na^+, K^+) dependent adenosine triphosphatase in the gills of the eel, *Anguilla anguilla*. Biochemical Society Transaction 3: 668.

Villarreal, B.W., P.M. Rosenblum, and L.T. Fries. 1994. Fatty acid profiles in red drum muscle: comparison between wild and cultured fish. Transactions of the American Fisheries Society 123: 194-203.

Volpe, J.J., and P.R. Vagelos. 1976. Mechanisms and regulation of biosynthesis of saturated fatty acids. Physiological Reviews 56(2): 339-417.

Warman, A.W., and N.R. Bottino. 1978. Lipogenic activity of catfish liver: lack of response to dietary changes and insulin administration. Comparative Biochemistry and Physiology 59B: 153-161.

Watanabe, T. 1982. Lipid nutrition in fish. Comparative Biochemistry and Physiology. 73B (1): 3-15.

Watanabe, T. 1993. Importance of docosahexaenoic acid in marine larval fish. Journal of the World Aquaculture Society 24(2): 152-161.

Watanabe, T., C. Ogino, Y. Koshishi, and T. Matsunaga. 1974b. Requirement of rainbow trout for essential fatty acids. Bulletin of the Japanese Society of Scientific Fisheries 40(5): 493-499.

Watanabe, T., F. Takashima, and C. Ogino. 1974a Effect of dietary methyl linolenate on growth of rainbow trout. Bulletin of the Japanese Society of Scientific Fisheries 40(2):181-188.

Watanabe, T., T. Takeuchi, and C. Ogino. 1975. Effect of dietary methyl linoleate and linolenate on growth of carp - II. Bulletin of the Japanese Society of Scientific Fisheries 41(2): 263-269..

Watanabe, T., T. Takeuchi, T. Arakawa, K. Sekiya, and C. Kitajima. 1989. Requirements of juvenile stripedjack *Longirostris delicatissimus* for n-3 highly unsaturated faty acids. Bulletin of the Japanese Society of Scientific Fisheries 55: 1111-1117.

Webster, C.D. and R.T. Lovell. 1990. Response of striped bass larvae fed brine shrimp from different sources containing different fatty acid compositions. Aquaculture 90: 49-61.

Williams, C.D., and E.H. Robinson. 1988. Response of red drum to various dietary levels of menhaden oil. Aquaculture 70: 107-120.

Wilson, A.C., and I.P. Williamson. 1970. Fatty acid synthetase from the liver of plaice, *Pleuronectes platessa*. Biochemical Journal 117: 26P-27P.

Wolters, B. 1987. Redfish farming. Proceedings of the Louisiana Aquaculture Conference. Louisiana State University, Agricultural Center, Baton Rouge. 110 pp.

Wurts, W.A., and R.R. Stickney. 1993. Growth rates of juvenile red drum *Sciaenops ocellatus* reared on commercial salmon feed in fresh and salt water. Journal of the World Aquaculture Society 24(3): 422-424.

Xu, R., S.S.O. Hung, and J.B. German. 1993. White sturgeon tissue fatty acid compositions are affected by dietary lipids. Journal of Nutrition 123: 1685-1692.

Yamada, K., K. Kobayashi, and Y. Yone. 1980. Conversion of linolenic acid to n-3 highly unsaturated fatty acids in marine fishes and rainbow trout. Bulletin of the Japanese Society of Scientific Fisheries 46(10): 1231-1233.

Yang, X., and T.A. Dick. 1994. Dietary α-linolenic and linoleic acids competitively affect metabolism of polyunsaturated fatty acids in arctic charr (*Salvelinus alpinus*). Journal of Nutrition 124: 1133-1145.

Yone, Y. 1978. Essential fatty acids and lipid requirements of marine fish. *In* Dietary Lipids in Aquaculture. Edited by Japanese Society of Scientific Fisheries, pp. 43-59. Koseisha-Koseikaku, Tokyo.

Yone, Y., and M. Fujii. 1975a. Studies on nutrition of red sea bream - XI. effect of n-3 fatty acid supplement in a corn oil diet on growth rate and feed efficiency. Bulletin of the Japanese Society of Scientific Fisheries 41(1): 73-77.

Yone, Y., and M. Fujii. 1975b. Studies on nutrition of red sea bream - XII. effect of n-3 fatty acid supplement in a corn oil diet on fatty acid composition of fish. Bulletin of the Japanese Society of Scientific Fisheries 41(1): 79-86.

Yu, T.C., R.O. Sinnhuber. 1972. Effect of dietary linolenic acid an docosahexaenoic acid on growth and fatty acid composition of rainbow trout (*Salmo gairdneri*). Lipids 7: 450-454.

Yu, T.C., R.O. Sinnhuber. 1975. Effect of dietary linolenic and linoleic acids upon growth and lipid metabolism of rainbow trout (*Salmo gairdneri*). Lipids 10(2): 63-66.

Yu, T.C., R.O. Sinnhuber and G.B. Putnam. 1977. Effect of dietary lipids on fatty acid composition of body lipid in rainbow trout (*Salmo gairdneri*). Lipids 12(6): 495-499.

Zar, J.H. 1984. Biostatistical analysis. Prentice-Hall, Inc., Inglewood Cliffs, New Jersey, USA.

APPENDICES

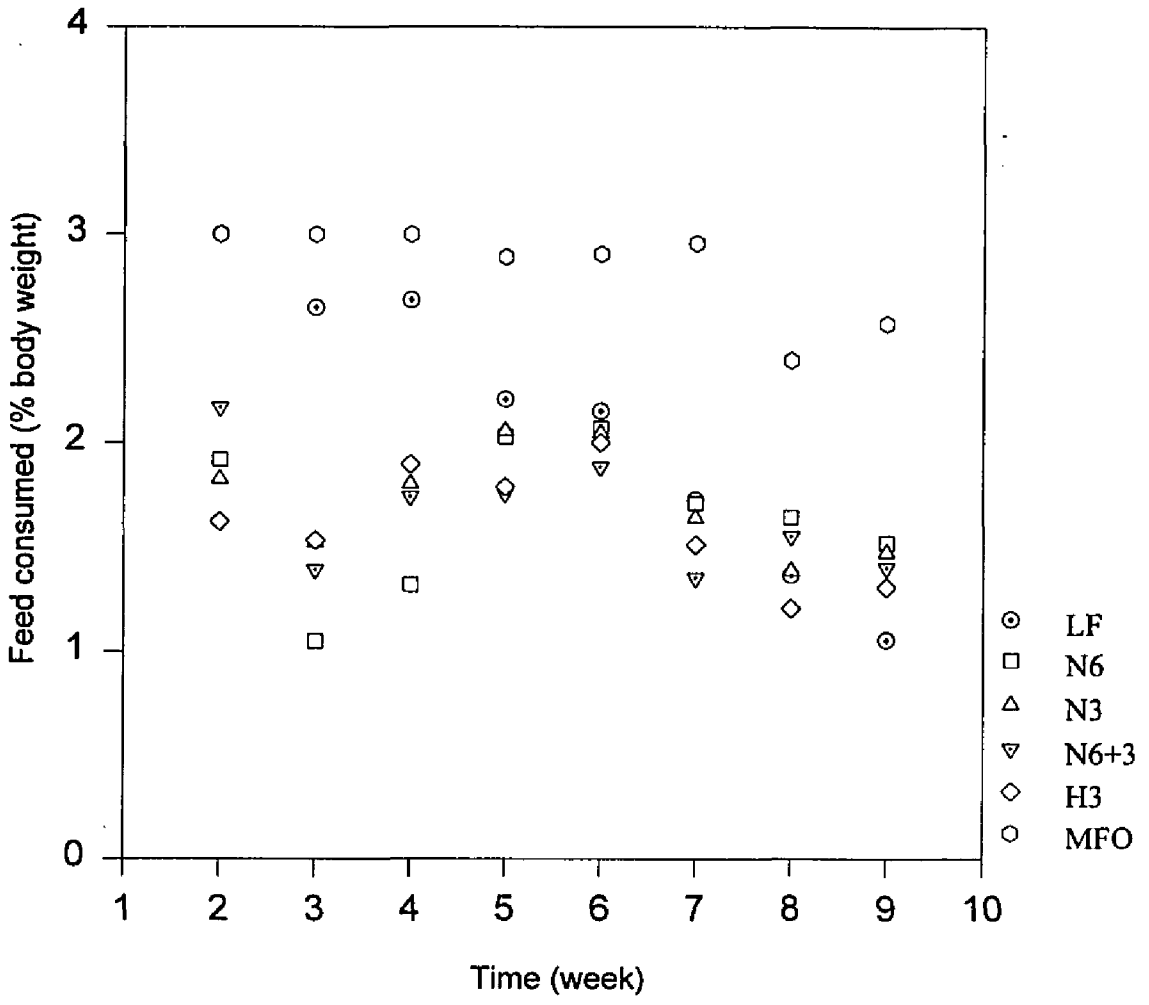


Figure A-1. Weekly consumption rate of red drum fed six experimental diets for nine weeks (3% body weight daily). Fish were fed 4% body weight in the first week (data not shown).

Figure A-2. Total saturated fatty acids in muscle polar lipid (MP), muscle nonpolar lipid (MNP), liver polar lipid (LP), and liver nonpolar lipid (LNP) of red drum fed six experimental diets for four and nine weeks. Means with same letter in the same fatty acid class in the same diet are not significantly different ($P > 0.05$). Means with same letter (with ') among dietary treatments of week four are not significantly different ($P > 0.05$). Means with same letter (with ") among dietary treatments of week nine are not significantly different ($P > 0.05$).

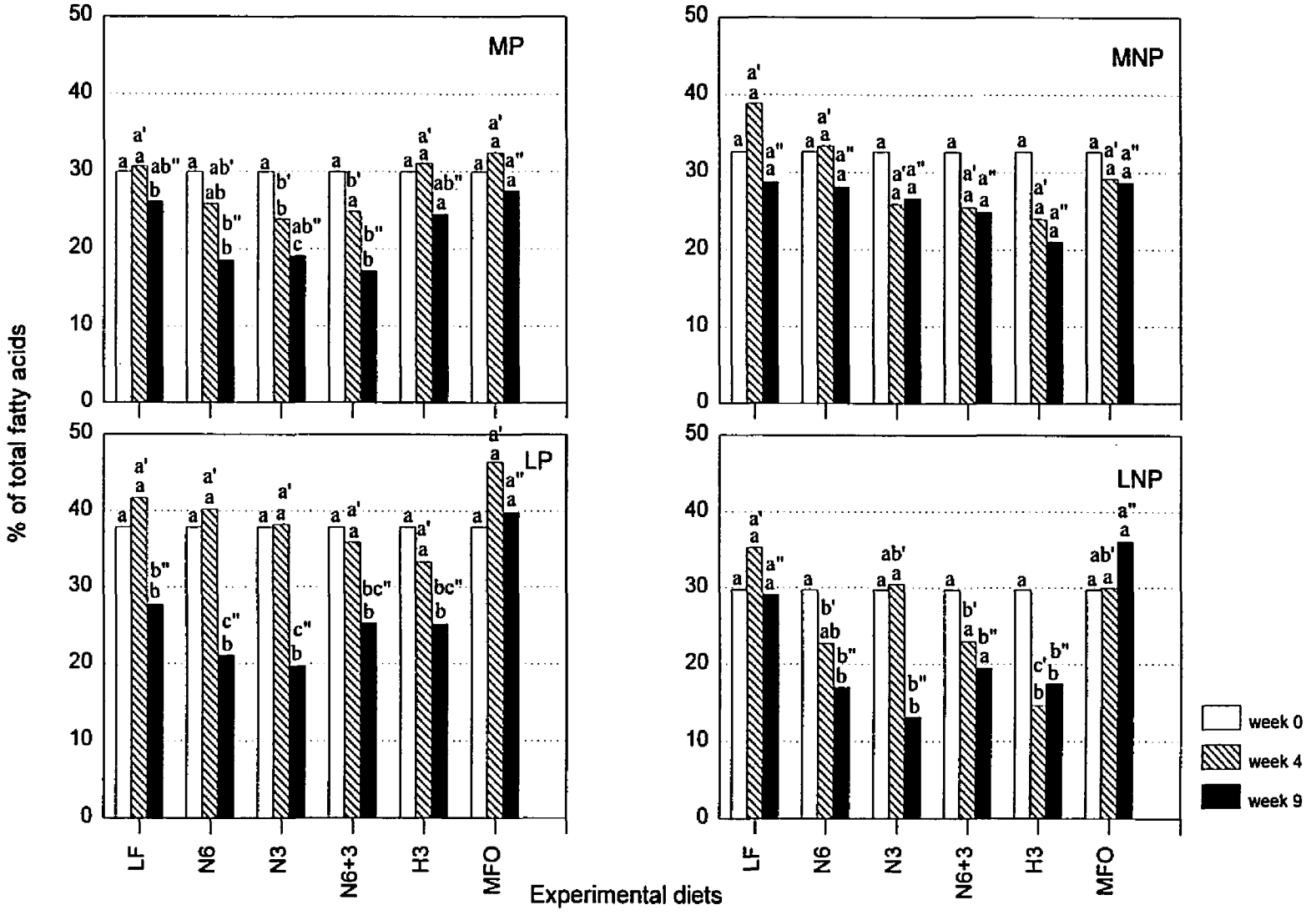

MP
MNP
LP
LNP
% of total fatty acids
Experimental diets
LF
N6
N3
N6+3
H3
MFO
week 0
week 4
week 9

Figure A-3. Total monoenoic fatty acids in muscle polar lipid (MP), muscle nonpolar lipid (MNP), liver polar lipid (LP), and liver nonpolar lipid (LNP) of red drum fed six experimental diets for four and nine weeks. Means with same letter in the same fatty acid class in the same diet are not significantly different ($P > 0.05$). Means with same letter (with ') among dietary treatments of week four are not significantly different ($P > 0.05$). Means with same letter (with ") among dietary treatments of week nine are not significantly different ($P > 0.05$).

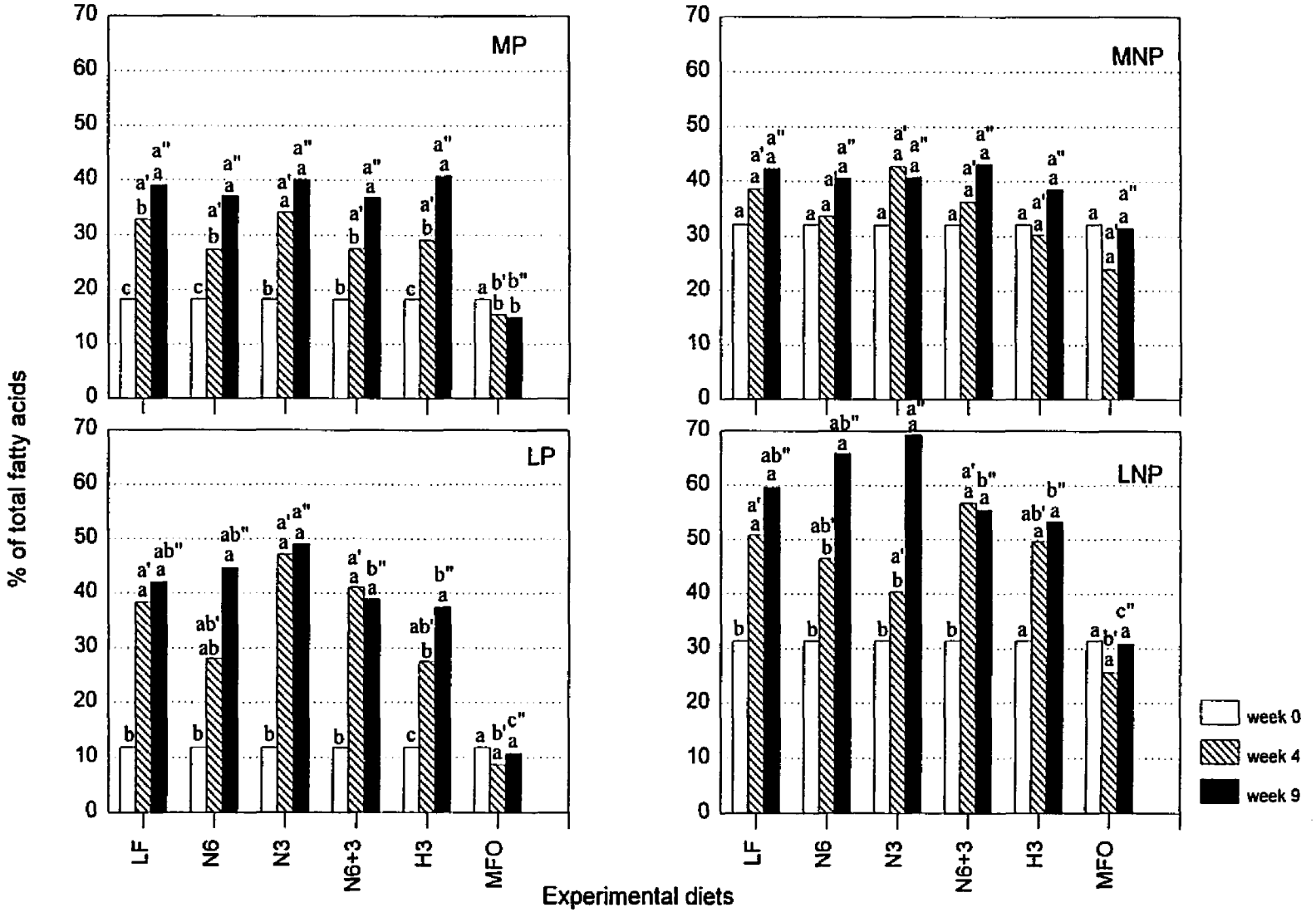

MP
MNP
LP
LNP
% of total fatty acids
Experimental diets
LF
N6
N3
N6+3
H3
MFO
week 0
week 4
week 9

Figure A-4. Total (n-3) PUFAs acids in muscle polar lipid (MP), muscle nonpolar lipid (MNP), liver polar lipid (LP), and liver nonpolar lipid (LNP) of red drum fed six experimental diets for four and nine weeks. Means with same letter in the same fatty acid class in the same diet are not significantly different ($P > 0.05$). Means with same letter (with ') among dietary treatments of week four are not significantly different ($P > 0.05$). Means with same letter (with ") among dietary treatments of week nine are not significantly different ($P > 0.05$).

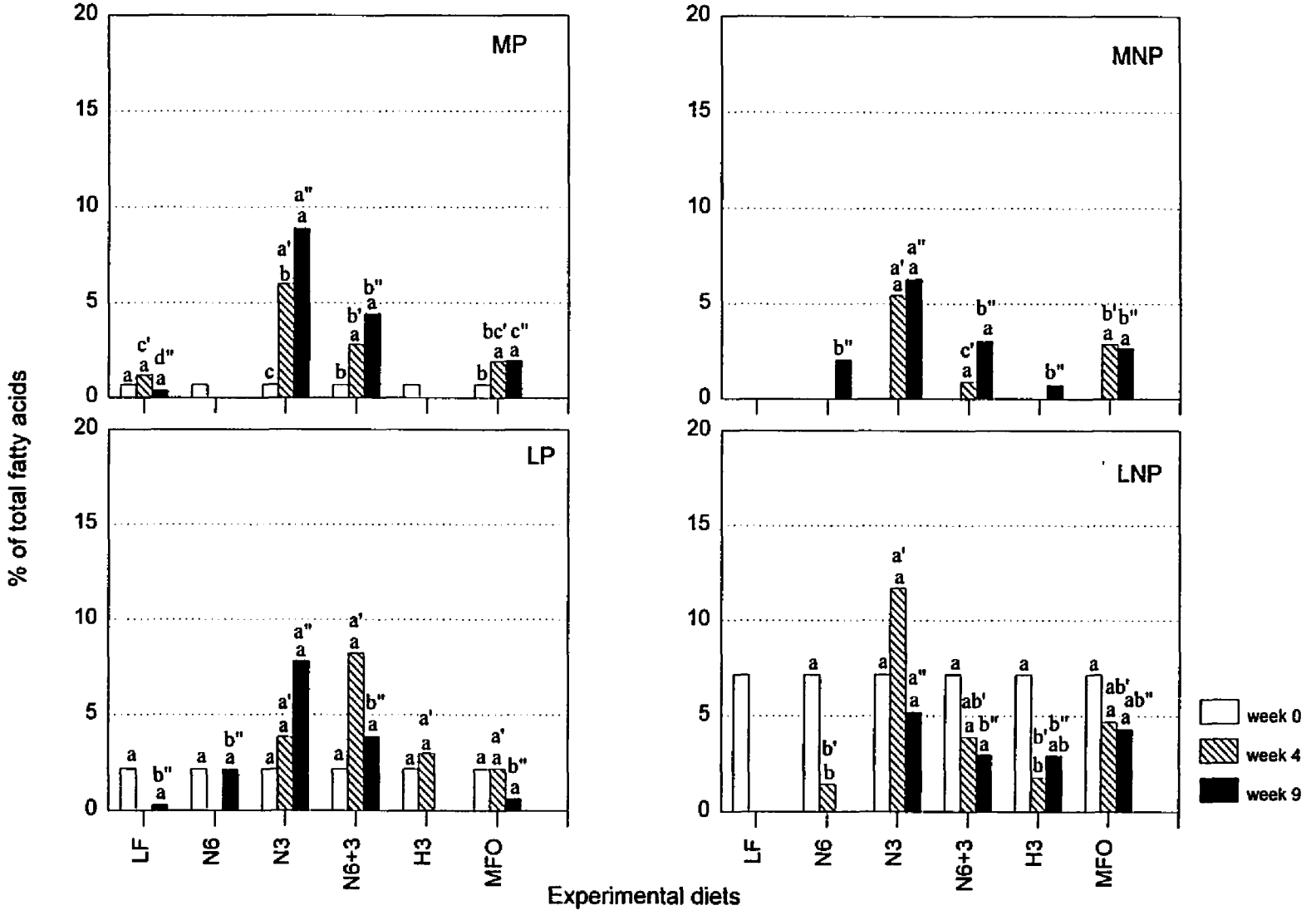

MP
MNP
LP
LNP
% of total fatty acids
Experimental diets
LF
N6
N3
N6+3
H3
MFO
week 0
week 4
week 9

Figure A-5 Total (n-6) PUFAs in muscle polar lipid (MP), muscle nonpolar lipid (MNP), liver polar lipid (LP), and liver nonpolar lipid (LNP) of red drum fed six experimental diets for four and nine weeks. Means with same letter in the same fatty acid class in the same diet are not significantly different ($P > 0.05$). Means with same letter (with ') among dietary treatments of week four are not significantly different ($P > 0.05$). Means with same letter (with ") among dietary treatments of week nine are not significantly different ($P > 0.05$).

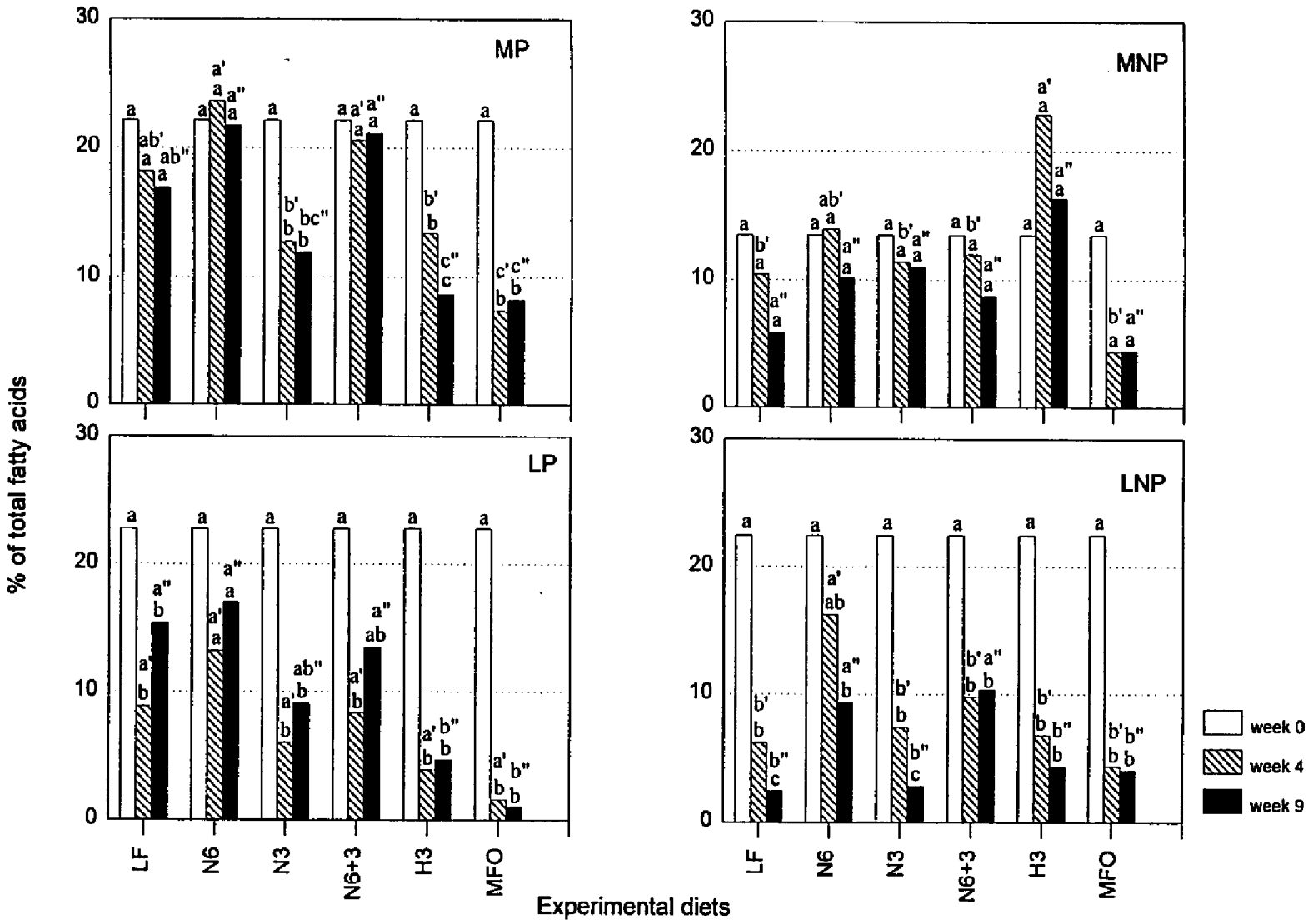
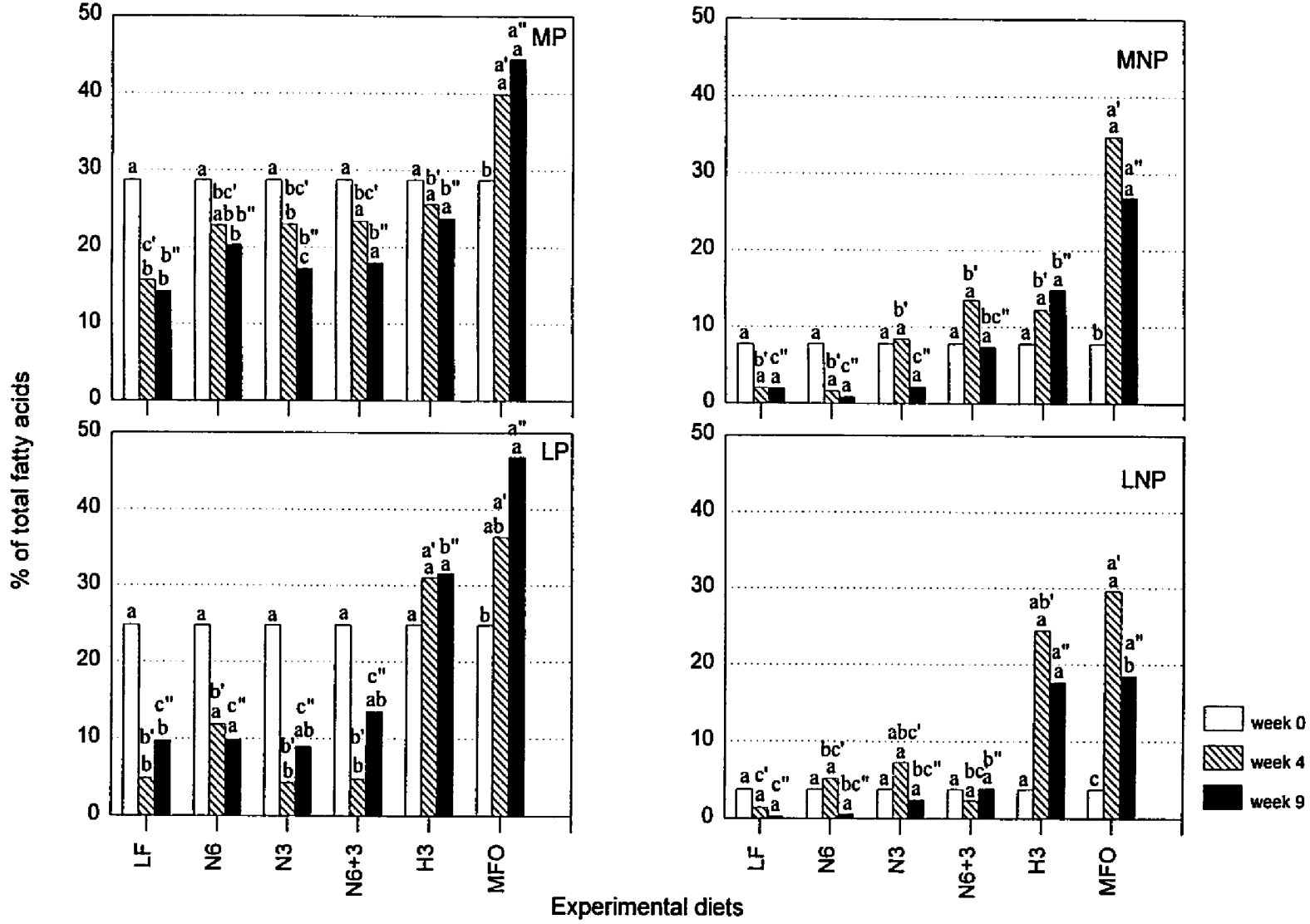

MP
MNP
LP
LNP
% of total fatty acids
Experimental diets
LF
N6
N3
N6+3
H3
MFO
week 0
week 4
week 9

Figure A-6. Total (n-3) HUFAs in muscle polar lipid (MP), muscle nonpolar lipid (MNP), liver polar lipid (LP), and liver nonpolar lipid (LNP) of red drum fed six experimental diets for four and nine weeks. Means with same letter in the same fatty acid class in the same diet are not significantly different ($P > 0.05$). Means with same letter (with ') among dietary treatments of week four are not significantly different ($P > 0.05$). Means with same letter (with ") among dietary treatments of week nine are not significantly different ($P > 0.05$).


MP
MNP
LP
LNP
% of total fatty acids
Experimental diets
LF
N6
N3
N6+3
H3
MFO
week 0
week 4
week 9

VITA

Carmen Chan is a Hong Kong born Chinese. She is currently enrolled in the Doctoral program at the Louisiana State University, School of Forestry, Wildlife, and Fisheries (commencement May, 1995) with emphasis on Fish Nutrition. She received her M.S. degree in Food Science from Auburn University in August 1989 and her B.S. degree in Psychology, with a minor in Mathematics from University of Wisconsin in May 1975. During her entire graduate studies, she supported herself either as a Graduate Teaching Assistant or Graduate Research Assistant. Carmen is also a member of the Institute of Food Technologists and the World Aquaculture Society.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Carmen Mayyuk Chan

Major Field: Wildlife and Fisheries Science

Title of Dissertation: Effects of Dietary Fatty Acid Ethyl Esters on Polar and Nonpolar Tissue Lipids in Red Drum, Sciaenops ocellatus

Approved:

Robert C. Reigh
Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

J. Samuel Godber

Robert P. Romaire

Date of Examination:

December 15, 1994