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Changes in Proximate and Fatty Acid Composition of Fillets from Channel Catfish during a Two-Year Growth Period

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Abstract.—Channel catfish *Ictalurus punctatus* were reared for 2 years on practical feeds with and without a topdressing of oil (50% animal, 50% vegetable). Visceral fat, fillet fat, and whole-body protein increased and moisture percentage decreased as fish size and age increased. Slopes of regression lines for these variables differed significantly between treatments. Fatty acid profiles of fillets showed essentially linear trends toward decreasing saturation and increasing unsaturation with increasing size and age of fish. Within the unsaturated fatty acids, monene levels increased, and diene and triene levels decreased. Topdressing with oil did not affect these trends. Although dietary lipid composition is a major influence on fatty acid composition in channel catfish, this study indicates that fish size and age also significantly influence fatty acid profiles.

Lipid levels in cultured channel catfish *Ictalurus punctatus* have been a continuing concern of the catfish processing industry (Lovell 1983). Fatter fish have lower processing yields (Stickney and Andrews 1971) and poorer storage quality because oxidative rancidity of fat is the major cause of product spoilage (Bjarnason 1979). Surveys have shown that average fat levels increased from 9% in cultured fish sampled in 1973 to 13% in fish sampled in 1982 (Lovell 1983).

Several factors have been implicated in the increase in lipid levels. These include increased feeding rates, harvest of larger fish, increased digestibility of energy sources in the feed due to feed extrusion (heat and pressure processing), spraying the feed with oil (topdressing) to reduce fines (dust), and changes in pond management (Avault 1985).

Although the total amount of lipid deposited is important, the fatty acid composition of the lipid is also important because it may influence flavor, odor, and storage characteristics (Ackman 1967; Worthington et al. 1972; Gatlin and Stickney 1982). A number of authors have shown that the fatty acid composition of many, if not all, fish species is a reflection of the fatty acid composition of dietary lipid (Worthington et al. 1972; Yingst and Stickney 1979). Worthington and Lovell (1973) showed that 93% of the variance in the fatty acid composition of channel catfish in their experiment was accounted for by diet. Reiser et al. (1963) reported that freshwater fish, marine fish, and probably other animals do not basically differ in their mechanisms for synthesis, interconversion, and deposition of fatty acids.

During digestion and absorption, dietary lipids (mainly triglycerides) are acted on by lipases, which cause the hydrolysis of triglycerides to free fatty acids (Church and Pond 1982). If not required metabolically, these free fatty acids can be re-esterified to form depot triglycerides. Through this process, a significant portion of the fatty acids present in the tissue lipids of fish can be derived unaltered from the food supply (Stickney and Andrews 1972; Dupree et al. 1979). However, profiles of deposited lipids can differ from those of dietary lipids depending on species (Church and Pond 1982), genetic variation (Yingst and Stickney 1979), metabolic need (Lovern 1964; Dupree et al. 1979), season (Gruger et al. 1964), and possibly age (Yingst and Stickney 1979).

The effects of several of these variables on lipid levels and fatty acid profiles of fish have been investigated. However, most nutrition work has been conducted with small fish in aquaria. Moreover, managerial and practical feed studies in ponds have usually been conducted over a relatively short period of time (one summer of growth).

The objective of our study was to determine the effects of practical diets with and without an added topdressing of oil on body composition and fillet fatty acid profiles of channel catfish from 6 to 30 months of age. This range includes most ages and sizes of fish encountered in commercial pond production of channel catfish. These data will be useful in making management, nutrition, and harvesting decisions.

Methods

We stocked young channel catfish (mean weight, 23 g) at 5,275 fish/hectare into four 0.04-hectare earthen ponds located on the Aquaculture Unit of

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the Mississippi Agriculture and Forestry Experiment Station near the Mississippi State University campus. Ponds were about 1.5 m deep and supplied with well water. Water temperature and dissolved oxygen were routinely monitored twice daily.

We fed fish in two ponds a 32%-crude-protein floating commercial catfish feed (Producer's Feed Company, Belzoni, Mississippi). Fish in two other ponds received the same feed top-dressed with oil (i.e., sprayed with feed-grade fat) of the type and at the rate (20 g oil/kg feed) being used by commercial feed mills in Mississippi. The topdressing oil was a hydrolyzed mixture of about 50% animal and 50% vegetable lipids (HEF, Buckeye Cellulose Corporation, Memphis, Tennessee), a feed additive commonly used in the poultry industry.

Eight batches of feed were used in the 2-year study. The feeds were analyzed by the Mississippi State Chemical Laboratory for crude protein, fat, fiber, ash, moisture, and gross energy. Nitrogen-free extract (NFE) was determined by difference (AOCS 1983; AOAC 1984). Crude fat of the feeds was determined by the acid-hydrolysis method (AOAC 1984). Gross energy was determined with a bomb calorimeter (AOAC 1984). Six batches of feed were analyzed for the fatty acid profile (AOAC 1984). Feeds were stored in bags at 1°C for no more than 3 months. Samples for analysis were taken at the beginning of the period when that batch of feed would be used.

At temperatures above 15°C, fish were hand-fed daily to satiation (as determined by feeding activity over a 20-min period), conditions permitting. When water temperatures were less than 15°C, feeding rates were based on water temperature, as recommended by Huner and Dupree (1984). Feeding time was at approximately 1500 hours each day, and feed was restrained within 2.5 × 2.5-m polyvinyl chloride feeding rings placed in each pond.

At the time of pond stocking and at quarterly intervals thereafter, we removed nine fish, representing the range of sizes present, from each pond. The fish were sexed, measured, and weighed. Visceral fat was removed and weighed. Skinned fillets representing the full length of the fish were removed and stored at −18°C in air-tight packaging. Within 4 weeks, proximate and fatty acid profile analyses were performed by the Mississippi State Chemical Laboratory (AOCS 1983; AOAC 1984). Lipid was extracted from the dried sample by AOCS method Ba 3-38 (AOCS 1983). Fatty acids were liberated and esterified by AOAC methods 28.053–29.056 (AOAC 1984). This procedure was slightly modified by replacement of the boron trifluoride reagent with a boron trichloride catalyst as the esterifying agent. Methyl esters were injected into the gas chromatograph in isooctane instead of hexane. Gas chromatography was used to separate and determine the methyl esters of the fatty acids (AOAC 1984: methods 28.057–28.065). Methyl esters were analyzed with a Hewlett-Packard 5710A gas chromatograph equipped with a flame ionization detector and a 2-m column of 3% of SP-2310 and 2% of SP-2300 on 100–200-μm-mesh Chromosorb (Supelco, Bellefonte, Pennsylvania). Quantitative results were based on a Hewlett-Packard 3380A integrator. Fatty acid methyl esters were identified by comparison with standards (Applied Science Laboratories, State College, Pennsylvania). Each acid was reported as a percentage of the total fatty acids.

Paired *t*-tests were used to compare proximate variables and overall mean values of individual fatty acids in the two feeds. Regression was used to examine fatty acid trends in the two feeds over time (Dowdy and Wearden 1983). We used Student's *t*-tests to compare mean values of weight gain, feed conversion, and survival (Dowdy and Wearden 1983). To investigate changes within each treatment over time, treatment means of percent moisture, protein, and fat in fillets, the percentage of total fatty acids in the saturated and unsaturated forms, and the ratio of unsaturated to saturated fatty acids (U/S) were regressed against sample period and tested for significance. Within the unsaturated fatty acids, the percentage of monenes (one double bond), dienes (two double bonds), and trienes (three double bonds) were also regressed against sample period and tested for significance. We evaluated treatment (addition of oil to the feed) effect on the above variables by using Student's *t*-test to determine significant differences in the slopes of the regression lines (Snedecor and Cochran 1980). If slopes were not significantly different, the full and reduced models were compared by the general linear test (Neter and Wasserman 1974) to determine if the data from the two treatments could be pooled.

Results and Discussion

Feeds

The percentages of protein and fiber in the feed were not affected by the addition of oil (Table 1). However, significant decreases ($P < 0.05$) in ash and nitrogen-free extract and significant increases

TABLE 1.—Proximate analyses (% wet weight) and energy contents (kJ/kg; mean ± SE) of two feeds fed to channel catfish during a 2-year study. Data are means ± SEs of eight batches; means within a row followed by the same letter are not significantly different (Student's *t*-test, $P > 0.05$).

Feed component or energy type	Feed without added oil	Feed with added oil
	Proximate analysis	
Protein	32.2±0.3 z	31.8±0.3 z
Fat	5.0±0.2 z	6.8±0.5 y
Moisture	8.6±0.7 z	9.9±0.5 y
Ash	7.0±0.1 z	6.6±0.1 y
NFE[a]	43.8±0.4 z	41.4±0.4 y
Crude fiber	3.5±0.1 z	3.5±0.1 z
	Energy	
Gross	17,991±16.2 z	18,410±33.5 y
Digestible[b]	11,811	12,473

[a] Nitrogen-free extract.
[b] Calculated from digestible energy values provided by Lovell (1989) and NRC (1983).

TABLE 2.—Fatty acid composition of the topdressing oil ($N = 1$) and mean compositions (±SD) of two channel catfish feeds ($N = 6$) as percentages of total fatty acids and ratios (%:%) of n-3 to n-6 fatty acids. There were no significant differences ($P > 0.05$) between feed treatments in mean levels of any of the fatty acids determined.

Fatty acids[a] or ratio	Oil	Unsupplemented feed	Oil-supplemented feed
Individual			
16:0	25.19	18.02±1.16	18.37±1.17
16:1, n-9	3.06	2.90±0.54	2.78±0.42
18:0	9.88	5.77±3.18	5.62±1.11
18:1, n-9	30.01	34.67±4.11	32.80±2.48
18:2, n-6	9.48	30.35±7.35	32.70±2.34
18:3, n-3	0.88	1.70±0.60	1.92±0.71
20:1, n-9	ND[b]	0.38±0.45	0.60±0.24
20:2, n-6	ND[b]	0.15±0.37	0.10±0.24
Others	21.50	6.06	5.11
Total			
Saturates	35.07	23.77	23.99
Monenes	33.07	37.95	36.18
Dienes	9.48	30.50	32.80
Trienes	0.88	1.70	1.92
n-3	0.88	1.70	1.92
n-6	9.48	30.50	32.80
n-3 : n-6 (%:%)	0.09	0.06	0.06

[a] Number of carbon atoms : number of double bonds, number of carbons from the methyl end where the first double bond occurs.
[b] ND = not detected.

($P < 0.05$) in fat, moisture, and crude energy were associated with feeds top-dressed with oil. Addition of oil increased crude fat level from 5% in the feed without oil to 7% in the top-dressed feed. This increased calculated digestible energy by an average of 6%.

Addition of oil to the feed had no discernible effect on fatty acid profiles of feeds used in the 2-year study (Table 2). This was probably due to the small amount of supplemental oil used. There were no significant differences ($P > 0.05$) between the two feeds in overall mean levels of unsaturated fatty acids, saturated fatty acids, U/S, monenes, dienes, trienes, or any of the individual fatty acids. Addition of oil did affect profiles of individual samples and oil-added feed tended to show less variation among the six feed batches than did unsupplemented feed. Apparent trends in feeds over the 2-year period were increased percentages of saturated fatty acids, decreased percentages of unsaturated fatty acids, and decreased U/S. Within the unsaturated fatty acids, monenes increased and dienes and trienes decreased. However, regressions of individual feed variables on time were not significant, and slopes for all except trienes were not significantly different from zero. Fluctuations were not due to intentional manipulated changes in diet formulations, but probably reflected seasonal variations in the sources and compositions of the ingredients themselves.

Fish growth.—There were no statistically significant differences ($P > 0.05$) between groups of channel catfish fed the two feeds over the eight quarters of the study in weight gain, feed conversion, or survival (Table 3). However, actual weight gain was less the first year (1984) in fish fed oil-supplemented feeds. Fish fed the feed without oil had an average weight of 431 g, 5% more than fish fed top-dressed feed (411 g). However, after the second growing season (third year of growth), fish fed feed without added oil had an average weight of 1,849 g, but fish fed oil-supplemented feed averaged 2,110 g. This represented a 14%

TABLE 3.—Mean (±SE) weight gain, feed conversion, and survival of pond-reared channel catfish fed commercial feed with and without a topdressing of oil over 2 years of growth (January 1984–January 1986). Each treatment was replicated twice. There were no significant differences ($P > 0.05$) for any variables between the treatments.

Feed treatment	Weight gain[a] (g)	Feed conversion[b]	Survival (%)
Unsupplemented	1,827.3±301.1	1.90±0.14	89.8±0.3
Supplemented	2,085.4±33.6	2.68±1.05	83.7±14.3

[a] Average starting weight was 23 g/fish for each treatment.
[b] Feed conversion = amount fed (g)/weight gain (g).

advantage in total weight for fish fed the top-dressed feed. The high overall feed-conversion ratios observed were probably due to the large sizes (>1,800 g) attained because feed-conversion efficiency decreases as fish size increases (Busch 1986).

The additional oil content of the top-dressed feed may possibly suppress growth in small fish. Young fish have a high protein requirement (as a percentage of the diet) for maximum growth (Piper et al. 1982). Wilson and Robinson (1982) recommended protein-to-energy ratios of 29–35 mg/kJ for diets based on practical ingredients. Without the added oil, the feed had a protein-to-energy ratio of 29 mg/kJ, which was reduced to 28 mg/kJ by the addition of oil. Page and Andrews (1973) also reported a reduction in growth for young channel catfish when fed diets containing elevated levels of fat. Hence, the 32% crude protein feed as formulated may have had a protein-to-energy ratio that was suboptimal for young, fast-growing channel catfish, especially when the energy was increased by the addition of the topdressing. In contrast, the protein-to-energy ratio in the unsupplemented diet may have been high for large fish and may have resulted in inefficient use of expensive protein, or even suppressed growth compared to that of fish fed the lower protein-to-energy (top-dressed) feed. However, current management techniques make it difficult to take advantage of size-specific feed formulations because most production ponds contain fish of several sizes.

Body Composition

Moisture.—Primary changes in the body composition of fish occur in moisture and fat (Groves 1970) because additional energy stored as fat by fish simply replaces body water (Reinitz 1983). Data from our study support these observations. The large decrease in moisture levels (Figure 1) coincided with a large increase in fillet fat levels. During 2 years of growth, fish in both treatments had a combined decrease of 8% in fillet moisture and a 4% increase in fillet fat. Regression of fillet moisture on time was significant in fish fed feeds with the topdressing ($P < 0.01$, $r = -0.94$) and without the topdressing ($P < 0.01$, $r = -0.92$; Figure 1). There was a significant difference ($P < 0.05$) in the slopes of the regression lines, and fish fed the supplemented feed showed a faster rate of decline in moisture.

Protein.—Most animals exhibit decreasing body protein levels over time (Church and Pond 1982). Reinitz (1983) has shown that protein increases with size and age in salmonids and that changes in moisture and fat levels do not adversely affect the deposition of protein. In our study, protein levels of fillets increased directly ($r = 0.91$) with increasing age and size of the fish (Figure 1).

The major exception to linear increases in protein was during the winter of 1985, when both treatments showed minor decreases in protein levels. These decreases corresponded to small increases in moisture. Regression of fillet protein on time was significant in fish fed top-dressed feed ($P < 0.01$, $r = 0.91$) and unsupplemented feed ($P < 0.01$, $r = 0.89$). The slopes of the regression lines were also significantly different ($P < 0.01$), indicating that fish fed the top-dressed feed had a faster rate of protein increase.

Visceral fat.—Fat deposited in the pleuroperitoneal cavity decreases dressed yield (Stickney and Andrews 1971) and represents waste to processors. Takeuchi and Watanabe (1977) recognized a positive linear relationship in carp between dietary lipid content and lipid in the muscle, viscera, and whole body; the lipid content of the viscera was affected most significantly.

In our study, the percentage of body weight contributed by visceral fat increased over the grow-out period (Figure 2), although seasonal fluctuations were evident. However, when actual visceral fat weights were examined (Figure 2), it appeared that visceral fat was not depleted substantially in winter, but remained essentially static. The decrease in winter percentage of body weight as visceral fat was due to slight increases in body weight (Figure 2), whereas actual visceral fat weights remained approximately the same.

Top-dressed feed fed to submarket-size fish (<500 g) had no significant effect ($P > 0.05$) on visceral fat deposition. A large increase in visceral fat of fish in both treatments occurred from April to July 1985, when the fish were ages 21–24 months, or at about the time of sexual maturity in channel catfish (Busch 1985).

Fillet fat.—Fish fed top-dressed feeds had consistently higher fat levels in their fillets than fish fed feeds without topdressing (Figure 1) after the age of 12 months (July 1984). Fillet fat differences became even more pronounced after fish reached sexual maturity (July 1985). The regression line for each treatment was significant ($P < 0.01$), and slopes were significantly different ($P < 0.01$). These regressions indicated that fish fed top-dressed feed had a significantly faster rate of increase in fat levels. Metabolic rates decrease after the fish attain sexual maturity (Phillips 1969), so the approximately 6% difference in digestible energy

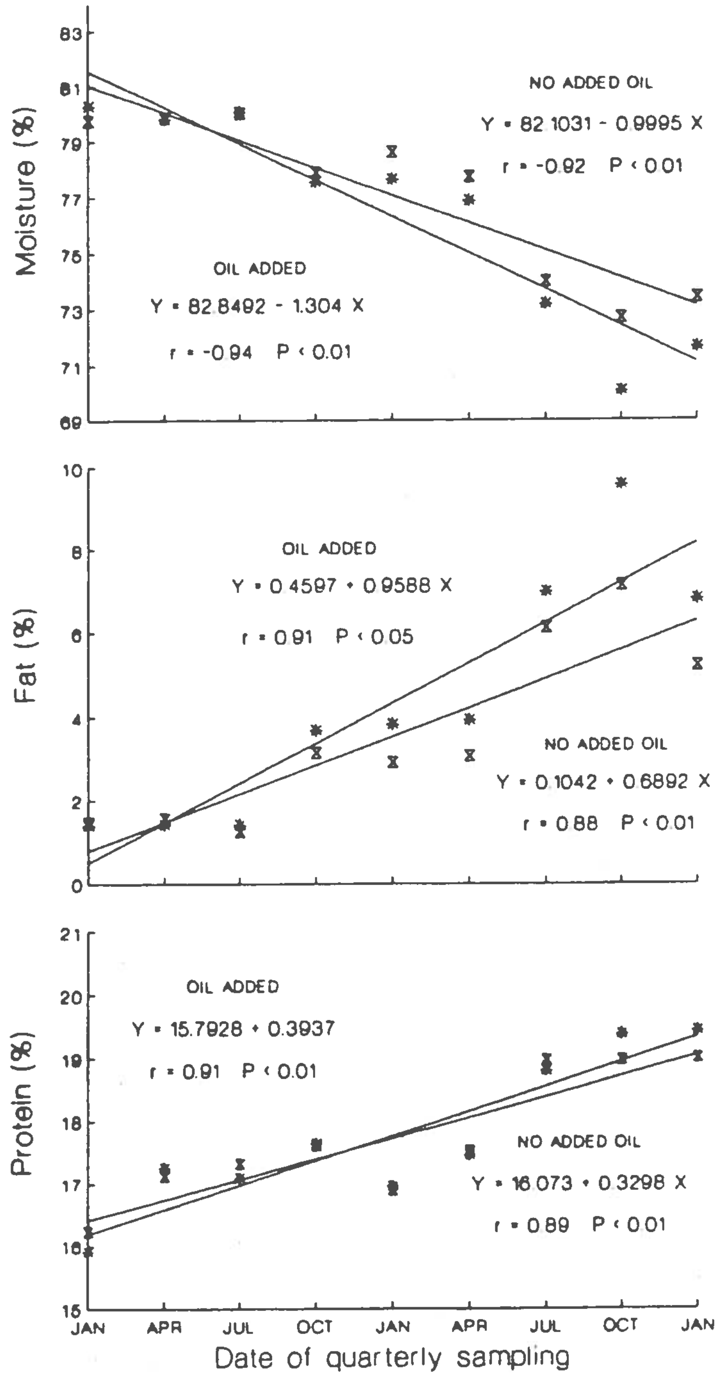

FIGURE 1.—Relationships between moisture (top), fat (middle), and protein (bottom) percentages (Y) and sampling date (X) in channel catfish fed commercial feed with (*) and without (⧖) an oil topdressing. Each treatment mean for the nine sample dates represents 18 fish.

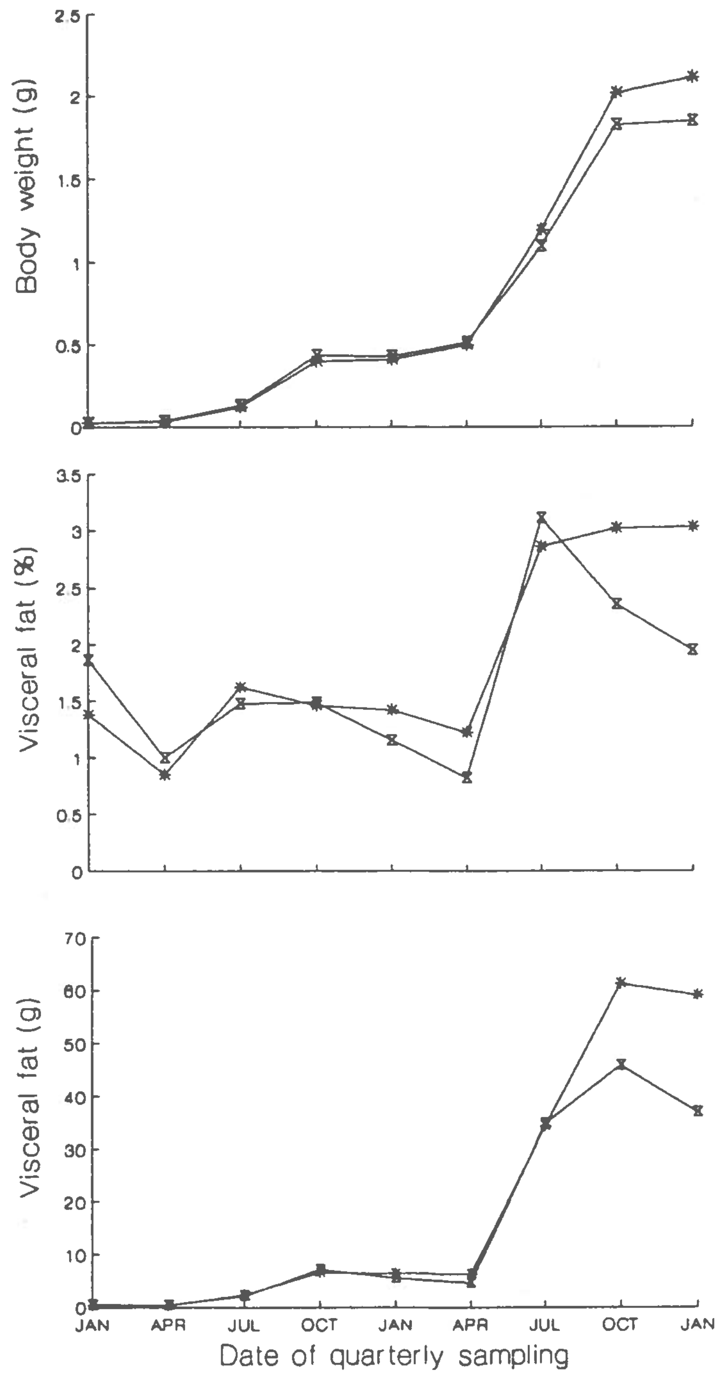

FIGURE 2.—Mean body weights (top), visceral fat as percentages of body weight (middle), and actual visceral fat weights (bottom) in channel catfish fed a commercial feed with (*) and without (⧖) an oil topdressing. Each treatment mean for the nine sample dates represents 18 fish.

levels in the feeds may have been sufficient to alter fat deposition. Differences in fillet fat (2–3%) between treatments may be sufficient to affect product quality and storability (Brooks 1982).

It is also notable that fat levels of fillets did not decrease in winter. The pattern of deposition was one of increase in warm months and then stability in cool months. However, these fish were on a

TABLE 4.—Mean fatty acid composition (±SE) of skinned channel catfish fillets (feed treatments pooled) as percentages of total fatty acids at stocking and at final harvest. Each mean represents 36 fish. For individual fatty acids, means along a row followed by the same letter are not significantly different (Student's t-test, $P > 0.05$).

Fatty acids[a] or ratio	Stocking	Final harvest
Individual		
16:0	21.69±0.45 z	18.15±0.34 y
16:1, n-7	4.52±0.35 z	4.81±0.52 z
18:0	5.93±0.71 z	3.72±0.45 y
18:1, n-9	37.59±1.17 z	51.29±2.13 y
18:2, n-6	17.75±0.48 z	13.19±0.40 y
18:3, n-3	3.68±0.29 z	0.77±0.34 y
20:1, n-9	2.17±0.03 z	1.39±0.16 y
20:2, n-6	ND[b]	ND[b]
Others	6.67	6.68
Total		
Saturates	27.62	21.87
Monenes	44.28	57.49
Dienes	17.75	13.19
Trienes	3.68	1.39
n-3	3.68	1.39
n-6	17.75	13.19
n-3 : n-6 (%:%)	0.21	0.11

[a] Number of carbon atoms : number of double bonds, number of carbons from the methyl end where the first double bond occurs.

[b] ND = not detected.

winter feeding program, and muscular fat might be mobilized in fish not fed during winter months.

Fatty Acid Composition of Fillets

Addition of a topdressing of oil to the feed had no effect on fatty acid profiles of fish fillets, even after 2 years of growth. There were no significant differences ($P > 0.05$) in the slopes of regression lines for any of the fatty acid variables of fillets from fish fed the two feeds. According to the general linear test ($P\alpha = 0.05$), there were no differences in treatments (in intercepts or slopes) among regression lines for any of the variables. Data for the two treatments were consequently pooled to give a single regression equation (Neter and Wasserman 1974). Although there were no significant effects due to the added oil, changes in fillet fatty acid profiles did appear over the study period. Using Student's t-test, we compared pooled stocking and final harvest means for individual fatty acids (Table 4). Of all the fatty acids analyzed, only palmitoleic acid (16:1, n-7; number of carbon atoms : number of double bonds, position of the first double bond counting from the methyl end) did not show a significant difference ($P > 0.05$).

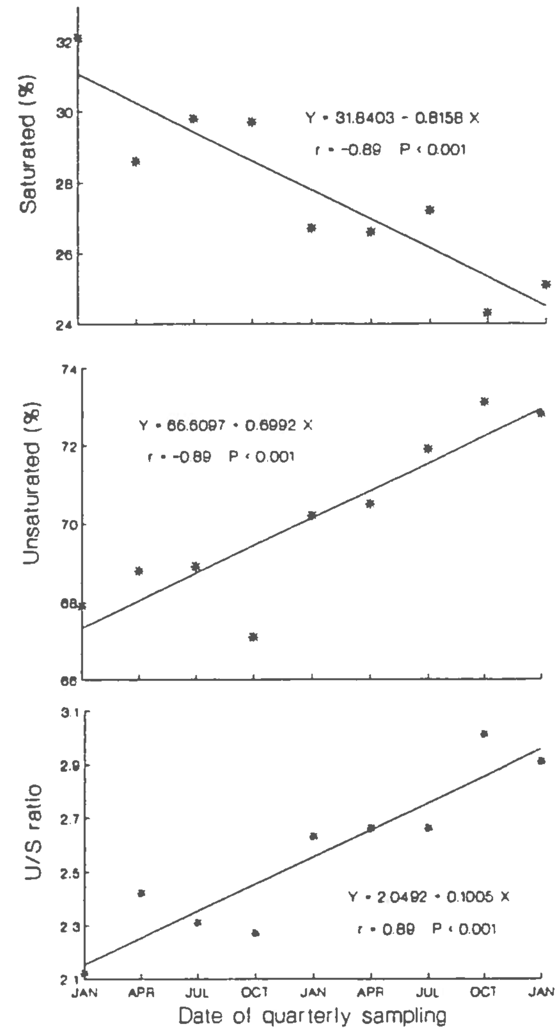

FIGURE 3.—Relationships between percent total saturated fatty acid (top), percent unsaturated fatty acid (middle), and ratio of unsaturated to saturated (U/S) fatty acids (bottom) in channel catfish fillets (Y) and sampling date (X). Each point represents a mean of 36 fish.

Saturated and unsaturated fatty acids.—During 2 years of pond culture, saturated fatty acid levels showed a linear decline from 32% of total fatty acids at stocking to 24% at harvest. Regression of saturated fatty acids on time (Figure 3) was highly significant ($P < 0.01$, $r = -0.89$). Accompanying this change was a concurrent significant increase ($P < 0.01$, $r = 0.86$) in unsaturated fatty acids, from 68% to 73% (Figure 3). The ratio of unsaturated to saturated fatty acids increased from 2.12 at stocking to a maximum of 3.01 at the sampling period just before final harvest. The regression of

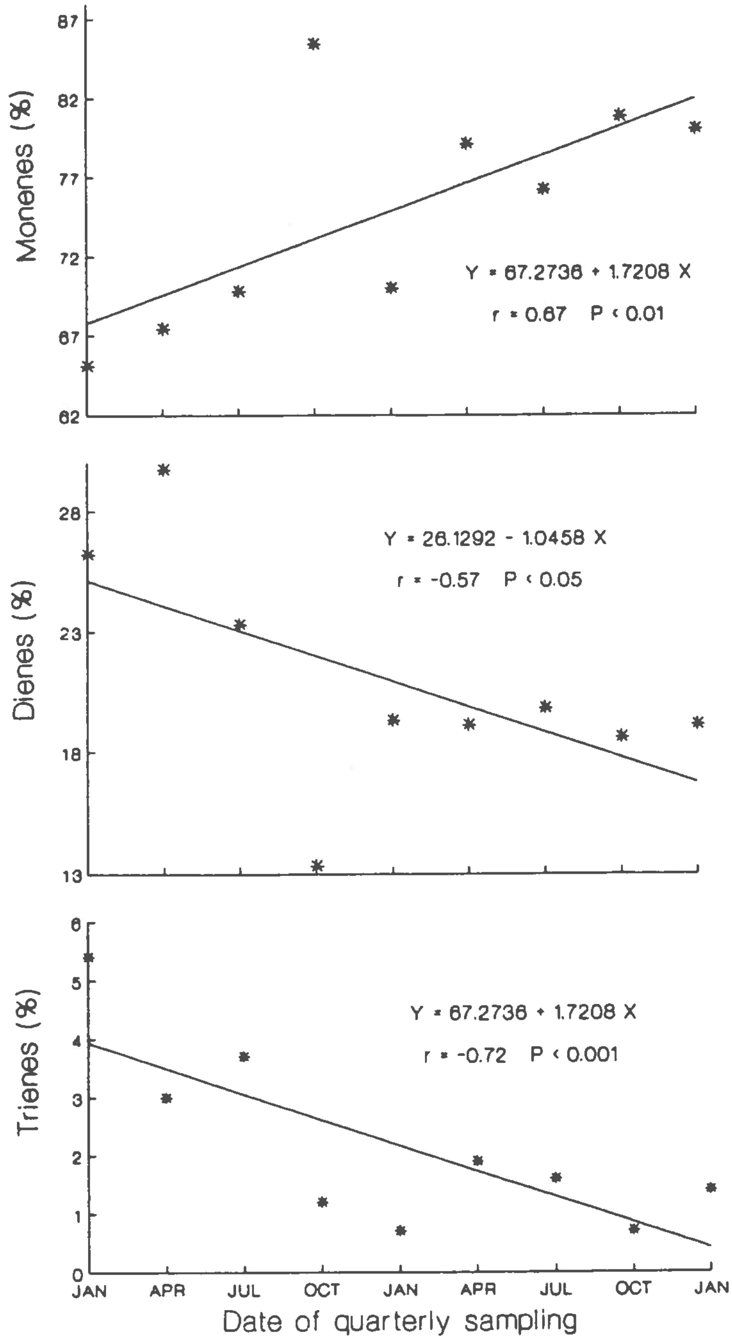

FIGURE 4.—Relationships between monenes (top), dienes (middle), and trienes (bottom) as percentages of total unsaturated fatty acids in channel catfish fillets (*Y*) and sampling date (*X*). Each point represents a mean of 36 fish.

U/S over time was highly significant ($P < 0.01$, $r = 0.89$; Figure 3).

Worthington and Lovell (1973) calculated that 93% of the total variance in carcass fatty acid composition was accounted for by diet. They did not, however, monitor changes over time. According to r^2 values for pooled treatments in this study, 79% of the variation in saturated fatty acids, 74%

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