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I am submitting herewith a dissertation written by Hussain M. Bakir (Al-Hakkak) entitled "Sensory Characteristics and Lipid Composition of White Amur (Ctenopharyngodon idilla) Fed Different Diets." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Technology and Science.

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SENSORY CHARACTERISTICS AND LIPID COMPOSITION OF WHITE AMUR (*CTENOPHARYNGODON IDELLA*) FED DIFFERENT DIETS

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Hussain M. Bakir (Al-Hakkak)

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DEDICATION

The author wishes to dedicate this work to his father and mother for their love and support.

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ABSTRACT

Two fish diets, trout chow pellets (A) and alfalfa pellets (P), were evaluated as to their effects on growth, lipid level and composition, fatty acid (FA) composition of polar and neutral lipids, and sensory characteristics of grass carp (Ctenopharyngodon idella).

During the 6 month feeding experiment, fish fed A gained 661 g/fish compared to 358 g for fish fed P. In addition, total lipid content in fish fed A increased from 1.84 to 3.72% in 4 months time, but remained approximately the same in fish fed P ($P < 1.0\%$) across feeding times. Three phospholipid classes: sphingomyelin (6 to 13%), lecithin (55 to 63%), and cephalin (25 to 35%) were affected by feeding time ($P < 0.05$). Nineteen FA were found in the phospholipids: 14:0, 16:0I, 16:0, 16:1, 18:0, 18:1, 18:1I, 18:2ω6, 18:3ω6, 18:3ω3, 20:1, 20:2, 20:3, 20:4ω6, 20:5ω3, 20:5ω6, 22:5ω3, 22:6ω3, and 24:1. Significant differences between diets were found in the percentages of 14 acids, and feeding time also significantly affected the levels of these acids. All of the previous FA except 16:0I plus 9 additional 16:1I, 17:0, 17:1, 20:0, 22:1, 23:0, 24:0, 24:4 and an unknown, were found in the neutral lipids. Significant differences between diets were found in the percentages of 18 acids in the neutral lipids, and feeding time significantly affected the levels of 19 of the acids. Compared with fish fed P, fish fed A had higher levels of monounsaturated FA (MONO) and lower levels of omega-6 polyunsaturated FA (PUFA) in both neutral and polar lipids; there were higher levels of saturated

FA (SAT) and omega-3 PUFA in the phospholipids but lower levels of SAT and omega-3 PUFA in the neutral lipids. Compared to P, A had lower percentages of SAT (40.9 vs. 51.1), omega-6 PUFA (12.1 vs. 18.2), and omega-3 PUFA (15.8 vs. 24.7) and a higher percentage of MONO (31.0 vs. 5.3).

There was no significant difference between the flavor of grass carp fed P (3.6) and that of catfish (3.7), but the panel (n = 93) scored the flavor of grass carp fed A (3.4) lower than that of catfish ($P < 0.06$). They also found no significant differences in texture among the three fish samples. Compared with catfish acceptability (3.6), grass carp fed P tended to have lower acceptability (3.4), but grass carp fed A a significantly lower acceptability (3.3). The main reason given for scoring the acceptability of grass carp lower than that of catfish was the presence of small bones in the fillets. This indicates that either grass carp must be bigger at market-size than those in this study (1585 g) or the bones must somehow be removed.

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CHAPTER I

INTRODUCTION

Grass carp (Ctenopharyngodon idella), also known as the white amur, is one of the largest members of the Cyprinidae. It has an elongated body with a relatively large head and terminally located mouth (Sneed, 1971). Unlike the common carp (Cyprinus carpio), grass carp lack barbels (Stevenson, 1965). The upper surface of the body is greenish-brown, while the sides and upper surface are silvery (Cross, 1969; Sneed, 1971). They are native to the rivers of Siberia, Manchuria, and China, which flow into the Pacific Ocean between latitudes 50N and 23N. They were introduced to the United States in 1963 from Malaysia to the Fish Farming Experiment Station in Stuttgart, Arkansas (Stevenson, 1965).

In almost every country where it has been introduced, the grass carp is cultivated for food. An exception is the United States where it is grown primarily for weed control (Cross, 1969). Since its introduction into the U.S., many aspects of the grass carp's biology have been investigated including food habits, potential as a cultivated food fish, and effectiveness as a biological control for nuisance aquatic vegetation.

Recently, the lipids in fish muscle have received much interest as a source of unsaturated fat in human diet regimen (Barlow & Stansby, 1982; Kinsella, 1987; Stansby, 1984). Data on lipid and

fatty acid composition of many fish and shell fish are available (Ackman & Takeuchi, 1986; Ackman, 1967a; Gibson & Worthington, 1977; Gopakumor, 1975; Halver, 1980; Joseph,1985; Mustafa & Medeiros, 1985; Stickney & McGeachin, 1984; Viswanthan & Gopakumar, 1984; Worthington et al., 1972; Worthington & Lovell, 1973).

The lipid in fish muscle can influence product quality through interaction with other components; therefore, after death the lipid in fish muscles undergoes two major changes. These are lipolysis and autoxidative deterioration of unsaturated fatty acids which result in product deterioration and undesirable aroma and flavors (Ackman, 1967b; German & Kinsella, 1985; Hardy, 1980). The effect produced by these changes are considered undesirable and are often the major problem in the frozen storage of some fish species. Therefore, lipid composition of fish and fish products is of considerable practical importance, particularly in relation to the effects of lipid components on deterioration during frozen storage and consumer acceptance (Ackman, 1967b; Anderson & Ravesi, 1968; Awad et al., 1969; Fukuda, 1955; Gibson & Worthington, 1977; Halver, 1980; Hansen, 1980).

In spite of the fact that grass carp is an important food source, research reports concerning the composition of grass carp, factors affecting composition, and the relationship between composition and fish quality are limited. More information concerning the effect of different diets on the growth, composition, and quality of grass carp is needed if the fish is to be utilized as a food source in the United States. Therefore, the objective of the present study

was to determine the effect of two diets, an animal-derived diet (trout chow pellets) and a plant-derived diet (alfalfa pellets), on the growth, lipid level and composition, fatty acid composition of neutral and polar lipids, sensory characteristics, and consumer acceptability of grass carp.

CHAPTER II

LITERATURE REVIEW

1. Grass Carp Natural and Artificial Feeding

Young grass carp begin feeding on zooplankton and phytoplankton shortly after emerging from the egg (Cross, 1969). Meske (1985) reported a 1969 study in which young grass carp weighing 4.14 g each were fed four different diets for a period of 3 months: (A) trout crumbs containing fish meal; (B) mixture of 75% Lucerne (alfalfa) meal and 25% soya meal (as crumbs); (C) chironomid (midge) larvae; and (D) Lemna minor (duckweed). The young grass carp grew best fed entirely on the animal diet (Diet C). Similar results have been reported by Bakir (1985) and Huisman (1978). De Silva & Wecrakoon (1981) observed that young grass carp are predominantly carnivorous in the wild even when vegetation is available as food.

Grass carp as ominovores have been noted by a number of researchers. Lin (1935) found they eat grass, leaves, and aquatic plants, as well as small fish, earthworms, silkworm pupae, flesh of fresh water mussels, beef, insects, and even decayed cloth and shoes. Stevenson (1965) reported that fingerlings fed heavily on Daphnia, chopped earthworms, and chironomid larvae. Cross (1969) reported that in his laboratory, grass carp of approximately 23 cm in length ate Daphnia, Tubifex worms, and Asellus, as well as vegetation. Singh et al. (1977) observed that small grass carp measuring

between 98 to 220 mm consumed mosquito larvae, and that fish up to a size of 185 mm were comparatively more voracious in feeding on mosquito larvae than the large fish (220 mm). They suggested that young grass carp 98 to 220 mm are capable of exercising some control over mosquito larvae. The larger grass carp of 252 mm length did not feed on the larvae.

In studies involving the grass carp as a cultured food fish, the diverse findings of grass carp feeding studies have suggested the use of several types of natural and artificial feeds. Shireman and co-workers (1977) reported that grass carp which were fed duckweed (Lemna minima) in circular tanks grew more rapidly at 0.53 fish/l than those stocked at higher densities. Shireman et al. (1978) used two different sizes of grass carp (3 and 63 g) and fed them one of four experimental diets: (1) Purina catfish chow pellets, (2) 50% catfish chow and 50% rye grass pellets, (3) 100% rye grass pellets, and (4) fresh duckweed. In this study, high biomass, excellent growth, and satisfactory survival rates were obtained when the fish were fed duckweed; the other diets were less satisfactory. Bakir (1985) studied the growth of two different sizes of grass carp (small, 21-37 g, and large, 400-729 g) in laboratory feeding experiments. The fish were fed three experimental diets including trout chow, bermuda grass pellets, and chopped sudan grass. Fish were fed at three feeding rates: 2.5, 5, and 10% of body weight. The results indicated that the fish fed trout chow pellets showed excellent growth whether they were small or large fish and whether or not they

were fed at the 2.5, 5, or 10% rate. In fish fed bermuda grass pellets, good growth was obtained only in large fish at the 2.5% level with better growth at 5%. Small fish lost weight when fed bermuda grass at 5% and 10%. The fish fed sudan grass at 2.5% body weight also lost weight during the six-week feeding trial.

Shireman et al. (1983) conducted an experiment to differentiate between grass carp and hybrid grass carp (grass carp ♀ x bighead carp ♂, Aristichthys nobilis). They were fed duckweed (Lemna sp.), hydrilla (Hydrilla verticillata), chara (Chara sp.), filamentous algae (Adogonium sp., Spirogyra sp.), and trout chow. They found grass carp grew best when fed hydrilla and trout chow and showed positive growth on all diets. Hybrid grass carp grew equal to grass carp when fed trout chow but did not grow when fed the vegetation diets. Crawford and Beadles (1980) fed alfalfa pellets (15% crude protein (CP)), pelleted catfish feed (30% CP), alfalfa and bermuda grass hay (10% CP), and freshly harvested vegetation (coontail, Ceratophyllum demersum, 11% CP, water smartweed, (Polygonium punctatum, and aquatic primrose, Jussiaca repens, 6% CP), to 31.8 to 36.3 g grass carp at 5% body weight per day. They found that the fish fed the catfish pellets or hay yielded poor growth (0.6 or 0.5 g gain/fish/week) and those fed alfalfa pellets grew faster (8.9 g gain/fish/week). They suggested that grass carp were unable to utilize a feed containing large amounts of animal protein; however, there was mortality throughout their study. Venkatesh and Shetty (1978) used two aquatic weeds (Hydrilla verticillata and Ceratophyllum

demersum) and terrestrial grass, the hybrid napier (a cross between elephant grass, Pennisetum purpureum, and sajje (Golgeri-1) P. typhoideum), as feed material for the grass carp. They found that growth attained by grass carp fed hybrid napier was nearly three times the growth gained with Hydrilla and about five times the growth gained with Ceratophyllum. Mgbenka (1983) evaluated three pelleted supplemental feeds in intensive feeding of grass carp in earthen ponds. The three feeds were commercial catfish ration and two modified foods in which dehydrated alfalfa meal comprised 19.3 and 38.5% of the catfish ration formula. The result of this study indicated that the fish grew satisfactorily on a commercial type catfish feed; the inclusion of 19.3% alfalfa meal in the formula improved growth slightly. There was no difference observed in fish fed the 38.5% ration and those fed the catfish ration without alfalfa meal.

2. Lipids and Fatty Acids Composition of Fish

According to Robinson and Wilson (1985), "Lipids are organic substances found in living matter which are insoluble in water but are soluble in non-polar solvents such as alcohol ether, chloroform, and benzene." Lipids of importance in fish and other animals can be classified as shown in Table 1.

Investigators have studied the distribution of specific phospholipids in tissues of aquatic invertebrates and in the muscle of fishes (Addison et al., 1968, 1972; Cossins, 1976; deKoning & McMullan, 1966; deKoning, 1966a, b; Gray & McFarlane, 1961; Patton,

Table 1. Classification of Lipids.

Type of Lipid	Example	Chemistry	Comments
Simple	Fats Oils Waxes	Esters of fatty acids with various alcohols	Most abundant lipids nature
Compound	Phospholipids	Esters of fatty acids containing groups in addition to an alcohol and fatty acid	Contain phosphoric acid
	Glycolipids		Contain carbohydrate
	Lipoproteins		Lipids bound to protein
Derived	Fatty acids Glycerol Other alcohols	Substances derived from other groups by hydrolysis	Usually contain an even number of carbons
Miscellaneous	Terpenes	Multiples of isoprene units	Vitamin A is an important terpene
	Sterols	Contain phenanthrene-type ring structure	Cholesterol, bile acids, sex hormones, Vitamin D, and cortisol are important sterols

Source: Robinson, E. H., & Wilson, R. P. 1985. Nutrition and feeding. In: Channel catfish culture: Developments in aquaculture and fisheries science, Tucker, C. S., ed. Elsevier-Amsterdam/Oxford, p. 323.

1975). All investigators found the most abundant phospholipids were phosphatidyl choline (lecithin) and phosphatidyl ethanolamine (cephalin). Sphingomyelin, phosphatidyl serine and phosphatidyl inositol were also present in small quantities. Nair and Gopakumar (1984) found similar results with five species of lean fish and three species of shellfish.

Fatty acids can exist as straight chain or branch chain components. Many of the fish fats contain numerous unsaturated double bonds in the fatty acid structures. A shorthand designation for fatty acid will be used throughout where the ω number identifies the position of the first double bond counting from the methyl end. Linoleic and linolenic acid would be written, respectively, 18:2ω6 and 18:3ω 3. The first number identifies the number of carbons; the second number, the number of double bonds, and the last number the position of the first double bond counting from the methyl end of the fatty acid.

The fatty acid composition of fish is influenced by many aspects. According to Ackman (1967b) and Castell (1979) as cited by Halver (1980), the fatty acid composition is affected by environmental influences like salinity as shown by the differences between the fatty acid compositions of marine and freshwater fish. Temperature also plays an important role in determining the fatty acid composition and especially the polyunsaturated fatty acid (PUFA) levels (Halver, 1980; Knippath & Mead, 1968). Diet affects the fatty acid composition of the fish. Worthington and Lovell (1973) indicated that the

differences in the composition of channel catfish (Ictalurus punctatus) fed six experimental diets were mainly related to diets (92.8%). Worthington et al. (1972) studied the fatty acid composition of channel catfish brought from five different processing plants located in Arkansas, Mississippi, Alabama and Georgia. They found the variability in fatty acid composition was due mostly to the diets. Kelly et al. (1958) reported that the differences between the fatty acid composition of the body fat of marine and fresh-water fish mostly resulted from differences in their dietary fatty acids. Stickney and Andrews (1971, 1972) and Yingest and Stickney (1979) studied channel catfish fingerlings which, when fed diets containing animal lipid such as beef tallow and menhaden oil, grew more rapidly than did those fed diets containing vegetable oils such as safflower oil and soybean oil with lipids comprising 10% of the diet. Stickney and McGeachin (1984) indicated that the decreased growth of channel catfish fingerlings fed vegetable oil may be a function not of essential fatty acid deficiency, but may instead result from the inhibition of de novo fatty acid synthesis, perhaps caused by excessive levels of dietary linolenic acid. Love (1970) showed that the fatty acid composition of monogastric animals, including aquatic and marine species of fish, is influenced by the fatty acid composition of the diet.

Lipids and fatty acid composition of fish and shellfish have been studied by many researchers. Viswanathan and Gopakumar (1984) studied lipid content and fatty acid compositions of five species of leanfish: silver jewfish (Johnius argentatus), milkfish (Chanos

chanos), pearl spot (Etroplus suratensis), catfish (Pseudarius jela and Tachysurus sp.), and three species of shell-fish: mussel (Perna viridis), carb (Neptunus pelagicus), and freshwater prawn (Macrobrachium rosenbergii). They found that phospholipid content of the leanfish varied from 0.7 to 0.89% and that of shellfish from 0.86 to 0.97% of the tissue. The phospholipid composition of fish and shellfish tissues did not show any variation from species to species. They found that phospholipids were richer in polyunsaturated fatty acids compared to neutral lipids, and that neutral lipids were richer in monounsaturated fatty acids compared with phospholipids. Gopakumar (1975) studied the fatty acid composition of three species of freshwater fishes: tilapia (Tilapia mossambica), barbus (Barbus carnaticus), and varal (Oplicephalus) by gas-liquid chromatography. He found that varal contained the highest amount of 20:5 acid compared to the other two species. The saturated fatty acid containing 17 carbons (17:0) was the predominant fatty acid in varal. Of all three fish, he found the highest percentage (23.3%) of 18:2ω6 acid in Barbus. Other researchers such as Hilditch and Williams (1964) observed that triglycerides from freshwater fish, compared to those of marine origin, were richer in fatty acids containing 16 and 18 carbons and lower in fatty acids containing 20 and 22 carbons. Pathak and Reddy (1962) and Bremner et al. (1961, 1963) found that tropical and subtropical freshwater species contained low levels of fatty acids with 22 carbons. Ackman (1967a) studied the fatty acid composition of oil from four North American freshwater fish (sheepshead, Aplodinotus

grunniens; tullibee, Coregonus artedii; maria, Lota lota; and alewife, Alosa pseudoharengus). He found that fatty acids containing 16 and 18 carbons were higher in these fresh- water fish than in marine species. In his study he observed that total di- and tetraenoic acids were three to four times as high in freshwater oils as in the marine oils. From his study, he suggested that extension of these to marine type fatty acids 20:5ω3 and 22:6ω3, etc. was not normally obligatory in freshwater fish.

3. Sensory Evaluation

Sensory evaluation uses the senses of taste, smell, touch, sight, and hearing to evaluate or measure physical properties of food (Amerine et al., 1965; Larmond, 1973,1977). The use of these responses has many applications in the entire research and development department in such areas as product matching, product improvement, cost reduction, quality control, and many other areas. Moreover, it plays an important role in new product development (IFT, 1981; Larmond, 1977; Tassan, 1980).

It has been reported that the ultimate determinants of cooked fish quality and consumer acceptance are flavor and texture (Boyd & Wilson, 1976; Laslett & Bremner, 1979). Several investigators recommended that the single most important factor in overall approval of fish seems to be flavor (Howgate, 1977; Lamb, 1975; Lindsay, 1980; and Rasekh et al., 1970,) identified intensity of off-flavor as the most influential factor affecting overall consumer preference of

yellow perch. However, in a study by Wesson and co-workers (1979) on the discrimination of quality of a variety of fish and fish products by inland consumer populations, the importance of fish texture to consumers was determined. When oxidized off-flavor or "fishy" flavors were low to moderate intensity, texture became an extemely influential determinant for preference. The authors concluded that texture should receive considerable attention in the assessment of consumer preference and acceptability of fish. They stated that textural aspects should receive equal attention with that of flavor in fish products.

Szczesniak (1972) reported that texture was cited by teenagers 25% more often than flavor as the reason for disliking fish and seafood. Cardello et al. (1982) hypothesized from multidimensional scaling of data derived from characterizing fish, that trained and consumer panelists used texture as one of the three determining dimensions in their evaluation processes. Texture of cooked fish is affected by many factors including species, age, size within species, nutritional state, and a wide range of environmental influences (Gibson & Worthington, 1977; Korschagen & Baldwin, 1971; Love, 1958, 1979a, b).

Few detailed studies have been reported on sensory testing methods for fresh or frozen fish (Mills, 1975). Although other authors have suggested that chemical and physical test results can be indicators of fish quality, sensory evaluation has been cited as the ultimate method for predicting consumer acceptability of fish (Bossey & Talabi, 1981; Shewan et al., 1953).

A consumer sensory test for evaluating cooked fish of blue whiting and whiting was conducted by Hamilton and Bennett (1981) using a 7-point hedonic scale for texture, flavor, appearance and acceptability. Samples were fried, steamed, or incorporated into fish pies. The results showed no significant differences among products or between fish types.

Grass carp have been widely scattered in many states in the United States as a biological control agent for nuisance vegetation in aquatic system (Guillory & Gasaway, 1978). Pflieger (1978) reported that grass carp have been evaluated by fishermen as a potential food fish because it is easy to clean, has a high percentage of usable flesh, and has a good flavor. Avault (1978) reported on informal taste tests on grass carp along with several other species including channel catfish and found that grass carp ranked first. Wilson and Cottrell (1979) conducted a taste panel and reported that grass carp was preferred less than channel catfish ($P<0.05$), but more than bluegill or largemouth bass.

Bakir (1985) showed that grass carp fed bermuda grass was preferred more than those fed trout chow ($P<0.05$). However, there was no significant difference in taste preference between grass carp fed trout chow or sudan grass and channel catfish. In the study of Bakir (1985), preference for a fish sample by a panelist was defined as those who scored the fish sample 4 or higher on a 6-point scale where 4 = like slightly and 6 = like extremely. Eighty-four percent of the panel preferred the taste of white amur fed sudan grass; 76% of

the panel preferred white amur fed trout chow, and only 71% of the panel preferred catfish. The indication is that white amur were more preferred than channel catfish.

4. Consumer Attitudes Toward Fish and Fish Consumption in the United States

Fish and fish products are significant food sources. The marketplace of seafood products and its availability have changed drastically in the past few decades. These changes came about because of improved processing capabilities and modern preservation methods. According to Lecos (1985), the USDA reported that the yearly consumption of fish has risen from 5.86 (1955) to 7.04 kg (1984) per person per year. According to Anon (1985), the 1983 annual per capita consumption of fish and shellfish in the United States was 5.17 kg (live weight equivalent). However, United States per capita consumption in the year 2000 is expected to reach 21.16 kg.

In the USA alone, some 500 different species have been marketed, and worldwide more than 1000 species have been marketed. Most species, however, are underutilized and only around 150 species are sold commonly to consumers in the United States (Martin et al., 1983; Stillings & Thompson, 1978). Displeasing nomenclature and consumer unfamiliarity with common and underutilized fish species have played a role in underusage.

Madeira and Penfield (1985) reported data generated from a consumer panel questionnaire. They found that the response to the

fish consumption was low and comparable to the average per capita level which is 5.58 kg per year. They also reported that just half of their panel prepared the fish at home and very few had prepared under-utilized species. From their study, they concluded that consumers needed more information and education with fish species and how to prepare them.

Lewis and Shoemaker (1986) studied the consumer attitudes and perceptions toward seafood when they ate out. They found that two-thirds of all seafood consumed in the United States is consumed away from home. They found that 84% of the consumers tested enjoyed going to restaurants; 82% ordered what they do not eat at home; 48% were concerned about the price, and 43% thought about nutrition. Lewis and Shoemaker (1986) found that there were 10 consumer attitudes toward fish which could be recognized. These were familiarity with the fish; consumers' disbelief that restaurant cooked fish was better than at home; religious and cultural beliefs; nutritional qualities; cost of fish; and the method of preparation and sauce that came with it.

Summary of Literature Review

As can be seen from this literature review, most of the reported research studied on grass carp have been concerned with finding the diets that would most effectively cause the fish to grow to stocking size (30 cm in total length), the size at which the fish begin to control aquatic weeds effectively. Reported research studies

on growing grass carp to market size are very limited. Reported investigations concerning the effect of diets on lipid concentration and composition, fatty acid composition and palatability characteristics of grass carp are even more limited. Additional research is needed to determine the most effective diet(s) for raising market-size grass carp with desirable palatability characteristics. Since fatty acid composition and lipid levels and composition have been closely related to flavor and even texture of food, the effect of these diet(s) on these chemical components also need to be determined. Economical production of a market-size grass carp with desirable flavor and texture might increase its market as a food fish and provide additional income to those who are using grass carp for aquatic weed control at the present time. Therefore, the objective of this investigation was to determine the effect of different diets on the size, lipid level and composition, fatty acid composition of the neutral lipids and phospholipids, and palatability characteristics and acceptability of grass carp.

CHAPTER III

METHODS

The fish used in this experiment were obtained from the TWRA Eagle Bend Hatchery in Clinton, Tennessee. They had been used in fish rearing ponds for algae control, and their average initial weight was 938 g.

A fish feeding experiment was conducted in the Food Technology and Science Laboratory located at The University of Tennessee, Knoxville, beginning 10 November 1985, using six circular tanks which were 122 cm in diameter and 73 cm in height. All tanks were fitted with venturi type drains and were covered with netting to prevent the fish from jumping out of the tanks. Water from a municipal source was dechlorinated using a carbon filter, and flowed through the tanks at a flow rate of approximately 2.5-liter/min. Before flowing into the tanks, the water was aerated by being deflected off polyethylene baffles. Compressed air was also injected into each tank to help aerate and maintain adequate dissolved oxygen levels at 8 ppm. Dissolved oxygen, chlorine, pH, and ammonia concentration levels in each tank were recorded at two-week intervals. All water quality tests were monitored by using the HACH DR-EL/1 portable testing kit. Water temperature was monitored in each tank throughout the experiment and was kept at 24±2°C.

Prior to the initiation of the experiment, the grass carp were acclimated to laboratory conditions for two weeks. Following acclimation, the fish were graded by size and assigned to each of the six tanks such that there was minimum fish size variation among tanks. Twelve grass carp were put in each tank. For Treatment 1, fish in three tanks were fed a diet of alfalfa pellets (Merry mixer), and for Treatment 2, fish in the other three tanks were fed a diet of trout chow pellets (Purina chow). These diets were fed at a rate of 2% of total body weight of each fish for each feeding time. Fish were fed twice a day (morning and late afternoon) for 6 days a week and for a 6 month period. Throughout this experiment a 12/12 hour light/dark photoperiod was used.

Sampling

Prior to the start of the experiment, three fish were selected at random for the 0 month samples. At two-month intervals throughout the experiment, the growth rate was determined by weighing all fish in the tank, and two fish were removed from each tank at 2, 4, and 6 months of feeding (a total of six fish per diet at each sampling time) for chemical analysis. Quinaldine was used to facilitate handling during the data collection periods.

In addition, representative samples of each diet were obtained for analysis.

Sample Preparation and Analysis

At the time of sampling, the head, viscera, and skin were removed from each fish. The remaining portion of each fish was wrapped in polyethylene coated freezer paper and stored at -18° C until analyzed.

Chemical Analyses

Within one month of storage, the fillets of each fish were removed and kept at room temperature for approximately 20-min prior to being cubed and placed in liquid nitrogen. After the cubes were frozen in liquid nitrogen, they were powdered in a high speed Waring blender equipped with 0.95-l stainless steel blender jar. The powdered sample was stored in sealed polyethylene bags (Whirlpak) at -18° C for not more than two weeks until analyzed for total lipid content, fatty acid composition of neutral and polar lipids, and phospholipid classes.

The diet samples were ground and analyzed for total lipid content and fatty acid composition of the total lipids.

Total Lipid Extraction and Analysis

Total lipids were extracted from 40 g of each powdered fish fillet sample and from 40 g of each diet. The chloroform-methanol method described by Melton et al. (1979) was used except that butylated hydroxytoluene (BHT, at .12 g/915 ml $CHCl_3$) was added to the extracting chloroform. The procedure was as follows:

1. Blend 40 g fish meat with 130 ml methanol for 5 min in a Waring blender at medium speed.
2. Add 65 ml chloroform containing 0.0085 g BHT and reblend 5 min.
3. Add 65 ml chloroform containing 0.0085 g BHT and reblend 20 sec.
4. Add 65 ml distilled water containing 1.5 g zinc acetate and reblend 10 sec.
5. Filter through Whatman No. 1 filter paper in a Buchner funnel with suction.
6. Reblend filter paper and residue with 100 ml chloroform containing 0.0130 g BHT for 2.5 min.
7. Filter as in step 5. Rinse blender with 75 ml chloroform containing 0.0098 g BHT and decant through filter.
8. Transfer filtrate to 500 ml graduated cylinder with 25 ml of methanol for rinsing.
9. Flush with nitrogen, cover and store at 5-6° C overnight or for 8 hrs.
10. Record volume of chloroform layer.
11. Pour contents of graduated cylinder into a 500 ml separatory funnel with a ground glass top and let stand at 5-6° C for 2-hours, to allow phase separation.
12. After 2 hours, drain bottom chloroform layer into a 500-ml round flat bottomed flask with a 24/40 ground glass top,

flush with nitrogen, stopper with ground glass stopper, and save for analysis.

Total lipid content in the fish or diet was determined in the following manner. For each sample, two 50 ml beakers were placed in the oven (100° C) for 30 min, cooled in a desiccator for 10 min and then weighed. Ten ml of the chloroform extract of each sample was evaporated from each beaker under a hood overnight. Then the beakers were placed in an oven at 95° C for 30 min, cooled in a desiccator for 10 min and weighed. Total lipid content in each sample was calculated from the equation of Melton et al. (1979) on an as-is basis. This equation is:

$$\% \text{ Total lipid} = \frac{(\text{lipid wt})\ (\text{total volume chloroform})}{(10 \text{ ml})\quad (40 \text{ g sample})} \times 100$$

After the duplicate 10 ml portions were taken from the lipid extract in the 500 ml flask, the flask was placed on a rotary evaporator, and its contents dried at 55° C under vacuum. The residue was dissolved in 25 ml of chloroform and stored at -18° C in a sealed 25 ml Erlenmeyer flask under nitrogen until analyzed for fatty acid composition and phospholipid classes.

Phospholipid Analysis

For each sample the concentration of total lipids in grams per ml of the concentrated lipid extract was determined in duplicate,

and 1.0 ml of this extract was diluted to 4 ml with chloroform and filtered through a 0.45 μm filter. Five-μl of this sample were analyzed for levels of the individual phospholipids (PL): cephalin, lecithin, and sphingomyelin, by a high performance liquid chromatographic (HPLC) method. The PL contents were determined for all fish fillet samples. The analysis was done on a Water's High Performance Liquid Chromatograph equipped with a Model 480 UV detector and a Shimadzu data processor (Model EIA) using a Whatman Partisil 10 column (length, 250 mm; o.d., 6.35 mm; i.d., 4.60 mm) and an acetonitrile: methanol:water (70:20:10; V:V:V) solvent containing 0.1% H_3PO_4 at a flow rate of 2.0 ml/min. The contents of phospholipids were measured at 205 nm. Percentage of each major phospholipid class: sphingomyelin, lecithin, and cephalin, was determined from the HPLC chromatogram of each fish, as follows:

1. Total = $A_1 + A_2 + A_3$

   where A_1 = area of the peak representing sphingomyelin,

   A_2 = area of the peak representing lecithin,

   A_3 = area of peak representing cephalin

2. % sphingomyelin = $\frac{A}{(\text{Total})} \times 100$, etc.

Determination of Fatty Acid Composition of Triglycerides and Phospholipids

Prior to the separation of phospholipids from neutral lipids, for each fish sample, two 50 ml beakers were placed in the oven for

30 min, cooled in a desiccator for 10 min, and weighed. One ml of the concentrated chloroform lipid extract from each sample was pipetted into each beaker, and the chloroform was evaporated from the beakers under hood for 3 hours. The beakers were then placed in an oven at 95° C for 30 min, cooled in a desiccator for 10 min, and weighed.

Determination of the volume of lipid extract needed to obtain 0.1 g lipid was obtained by the following equation:

$$\text{X ml of sample} = (0.1 \text{ g of lipid}) \frac{1}{\text{lipid concentration, g/ml}}$$

After the determination of the milliliter of lipid sample and prior to fatty acid analysis of phospholipids for each sample, phospholipids were separated from neutral lipids on a Water's silica Sep-Pak in the following manner:

1. Dry the lipid extract on a rotary evaporator under vacuum at 55° C.
2. Add 10 ml chloroform to the lipid residue in small flask. Swirl to dissolve.
3. Attach silica Sep-Pak to a 10 ml syringe. Pass 10 ml chloroform through Sep-Pak.
4. Pass 9 ml of lipid solution from #1 through Sep-Pak. This goes into 125 ml flask labeled "Triglyceride."

5. Pass 10 ml of 7% petroleum ether in ethyl ether through Sep-Pak. Add this to triglyceride flask.
6. Pass 40 ml of methanol through Sep-Pak (10 ml at a time). Collect this in flask labeled "Phospholipid."
7. Add 1 ml of butylated-hydroxy-toluene (BHT) solution to (PL) flask (use 0.01% BHT in methanol).
8. Add accurately one milliliter of internal standard solution (1 mg/ml solution of nonadecanoic acid in methanol) to each PL flask and store sealed under N_2 at -18° C until fatty acid analysis.

Prior to fatty acid analysis of the phospholipids, the PL fraction was dried to dryness in a 125 ml Erlenmeyer flask with a 24/40 ground glass top under vacuum using a rotary evaporator at 55° C. The fatty acids of the dried phospholipids were converted to methyl esters by the boron trifluoride method of AOCS (1974) as follows:

1. Four ml of 0.5 N methanolic sodium hydroxide and boiling beads were added to the dried phospholipids in the 125 ml reaction flask.
2. The flask was attached to a water-cooled condenser, and its contents refluxed for 10 min.
3. Five ml of BF_3-methanol reagent (Supelco Inc., Bellafonta, PA) were added to the reaction flask through the condenser, and its contents boiled for 2 min.

4. The reaction flask was cooled before 7 ml of pentane were added to its contents through the condenser, and the contents of the flask refluxed for an additional minute.
5. The reaction flask was removed from the heater and cooled before being disconnected from the condenser.
6. Enough saturated NaCl solution was added to the flask to float the pentane solution containing the methyl esters in the neck of the flask.
7. Five to seven ml of the pentane solution containing the methyl esters were transferred into a test tube, and a small amount of anhydrous sodium sulfate was added to dry the pentane solution. This water-free pentane solution was concentrated to 0.5 to 1.0 ml total volume under a flow of N_2 and 1 to 3 µl were analyzed by a gas chromatographic (GC) method using a 30 meter by 0.25 mm i.d. fused silica SP-2330 capillary column (Supelco Inc., Bellafonta, PA). The GC run was temperature programmed from 150 to 220° C at 2° C/min on a Shimadzu Model 6AM gas chromatograph equipped with a flame ionization detector and a Shimadzu E-18 data processor. Selected fatty acid methyl ester samaples were also analyzed on the same capillary column using a Shimadzu Model 9 am gas chromatograph (GC) interfaced with a Shimadzu QP-1000 mass spectrometer (MS) set at 70 ev for fatty acid identification. The temperature program for the GC-MS run was 150°to 188° C at

2° C/min, followed by a rate of 5° C/min from 188 to 220° C. Tentative identification of individual compounds in each sample was determined by comparison of their retention times from GC analysis with those of known standards. Positive identification was made by matching the mass spectrum of the fatty acid in the sample with that of a standard known fatty acid. The standard containing known concentrations of the major fatty acids, similar to concentrations present in the sample, was analyzed in the GC method. Percentage of each fatty acid present in the phospholipid was calculated as described by the AOCS (1974) method. After phospholipid fatty acid composition had been determined, several triglyceride or neutral lipid fractions were checked by the previously described HPLC method for presence of phospholipids, and all samples contained trace amounts of the phospholipids. Because of this, neutral lipids were separated from phospholipids for each sample again using a modified elution procedure from a Water's Sep-Pak.

The weight of lipids (g/ml) in the total lipid concentration extract of each sample was determined, and a volume (ml) containing approximately 90 mg was removed from each extract and dried under N_2 flow. The dried residue was dissolved in 1 ml of petroleum ether; ethyl ether, 92:8, V/V, and loaded on a Water's Silico Sep-Pak

attached to a 10 ml disposable plastic syringe. Triglycerides on neutral lipids were eluted from the Sep-Pak by 40 ml of petroleum-ether, 92:8, V/V. Since BHT eluted with the neutral lipid, it was not necessary to add additional BHT to protect against oxidation. After the neutral-lipid fractions were checked by the HPLC method for presence of phospholipid and found to contain none, the fatty acid composition of each neutral lipid fraction was determined in the same manner as described for that of the phospholipids starting with the addition of 1 mg of nonadecanoic acid to the triglyceride fraction to standardize retention times of all acids.

In addition to determining the percentage of each fatty acid in the polar and neutral lipids, the total percentage of the following fatty acid types: saturated, monosaturated, polyunsaturated omega-6 (Omega-6) and polyunsaturated omega-3 (Omega-3), were also determined. For the polar lipids, the saturated fatty acid type included 14:0, 16:0, 16:0I, 17:0 and 18:0; the monosaturated, 16:1, 17:1, 18:1, 18:1I, 20:1 and 24:1; Omega-6, 18:2ω6, 18:3ω6, 20:2, 20:3, 20:4ω6 and 20:5ω6; and the Omega-3, 18:3ω3, 20:5ω3, 22:5ω3 and 22:6ω3. For the neutral lipids the saturated fatty acid type included 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 23:0 and 24:0; the monounsaturated type included 16:1, 16:1I, 17:1, 18:1, 20:1, 22:1 and 24:1; the Omega-6 type included 18:2ω6, 18:3ω6, 20:4ω6, 22:5ω6, 20:2, 20:3 and 24:4; and the Omega-3 type included 18:3ω3, 20:5ω3, 22:5ω3 an 22:6ω3.

Lipid Level and Fatty Acid Composition of the Diets

The levels of total lipids in the diets were determined by the same procedure that was used to determine the levels in the fish fillets, and the fatty acid composition of the total lipids of each diet was determined in the same manner as the fatty acid composition of the fish phospholipids.

Sensory Evaluation

Upon termination of the feeding experiment, grass carp fed the two diets (alfalfa and trout chow pellets) were subsequently skinned and fillets were removed from each fish and stored wrapped in polyethylene-coated freezer paper at -18° C for approximately 3 months until analyzed. Refrigerated channel catfish fillets were purchased from a local supermarket just prior to sensory analysis to use as a comparison sample.

All samples were prepared for taste panel assessment according to the methods of Wilson et al. (1973). Organoleptic properties of the grass carp from each treatment and channel catfish were evaluated by a 93-member taste panel at one time. Deep-fried portions of fish fillets from each diet were assigned a three-digit code number which had been randomly selected in order to eliminate bias. Grass carp samples from each treatment and the catfish sample were served, one at a time under white lights; each panelist received a score card with each sample. The score card, which is shown in Appendix A, had three attributes listed: flavor, texture, and overall acceptability.

Each panelist marked how he/she felt about each attribute for a single sample by crossing one of five faces which progressed from an unhappy expression to a happy grin for each attribute.

Panelists were instructed also to rinse their mouths between samples. The five faces represented a five-point hedonic rating scale as follows: 5 = very positive feeling or the happiest face; 4 =like moderately, positive feeling; 3 = like slightly or slightly positive feeling; 2 = dislike slightly or slightly negative feeling; 1 = dislike moderately or moderately negative feeling or the unhappiest face. After the third sample, a questionnaire (Appendix B), was distributed to each panelist for a demographic information study, frequency of eating fish, and familiarity with different types of fish.

Statistical Analysis

Each dependent variable (% fatty acid in phospholipids or neutral lipid, % total lipid in fish, and % phospholipids class) was statistically analyzed as a function of treatment, feeding time, and treatment by feeding time interaction. If the interaction were not significant at the $P<0.10$ level, then feeding time effects ($P<0.10$) were separated across treatments by orthogonal polynomials into linear, quadratic and cubic effects. In the event of a significant interaction, significant feeding time effects were separated for each treatment into linear, quadratic and cubic effects. Significantly different means between treatment did not need to be separated since there were only two treatments.

Sensory analysis results (flavor, texture, and acceptability) were analyzed as a function of sample type (grass carp fed trout chow, grass carp fed alfalfa pellets or catfish), and panelist. Significantly different means ($P < 0.05$) among types of samples were identified by the student Newman-Keuls test.

All data were analyzed statistically by general linear models using Statistical Analysis Systems (SAS) reported by Barr et al. (1979).

CHAPTER IV

RESULTS AND DISCUSSION

Chemical Analysis of Water and Temperature Data

Chemical analysis of water revealed that ammonia, pH, and chlorine levels did not differ significantly among treatments during the experiment (Table 2). According to Knepp and Arkin (1973) the ammonia level should be below 30 ppm for catfish to avoid symptoms of ammonia poisoning. According to Worsham (1975), the National Technical Advisory Committee (1968) reported that the recommended potable water limit for ammonium -N is 0.5 ppm and ammonium -N greater than about 2.5 ppm can be toxic. Those results gave an indication that the ammonia levels in this study (1 µg/ml) were well within tolerance limits determined for other fishes. Dissolved oxygen levels were also within acceptable ranges throughout the experiment. Shireman et al. (1977) and Bakir (1985) reported similar results for the oxygen levels when assessing the effect of feed consumption rates and stocking densities of grass carp on dissolved oxygen concentration in tanks. The most probable reasons for the lack of fluctuation among chemical parameters may be the turnover rate in the tanks (2200-3300 ml/min), the cleaning of residues from the bottom of the tank every weekend, and the complete cleaning of each tank every two months. Bakir (1985) found similar results when he studied the effect of different diets on the growth of grass carp in circular tanks. The

Table 2. Mean Water Quality Parameters as Determined for Water Collected From Each Feeding Trial.

Diet	Temp. (°C)	Oxygen[a] (µg/ml)	pH	Ammonia/ Nitrogen (µg/ml)	Chlorine (µg/ml)
Alfalfa	24 ±2	8 ±1	7	1	<0.5
Trout chow	24 ±2	6 ±1	7	1	<0.5

[a]Type water dissolve oxygen 11 (µg/ml).

temperature levels (23 to 27° C) also were within the optimum range for grass carp growth as reported by Russian workers (Anon, 1970).

Fish Growth and Lipid Composition

There was a significant difference in weight gain between grass carp fed trout chow and those fed alfalfa pellets. Fish fed trout chow outgrew those fed alfalfa pellets (Figure 1). During the 6 months feeding period when the diets were fed at 2% body weight, the fish fed trout chow gained an average of 661 g compared with 358 g for the fish fed alfalfa pellets. The difference between the weight gains may be attributed to differences in diet composition; thus, the quality of the diet food plays an important role in fish growth. Bakir (1985) reported the growth of two different sizes of grass carp which were fed three experimental diets (trout chow, bermuda grass pellets, or chopped sudan grass) at three feeding rates: 2.5, 5 and 10% of body weight. Both sizes of fish fed trout chow at all three feeding rates showed excellent growth; however, bermuda grass produced good growth only in large fish, and fish fed sudan grass lost weight. The present study is in agreement with Bakir (1985) in that the animal diet (trout chow) produced better growth than the plant diet (alfalfa pellets).

Total lipid levels in fish on both diets and the composition of the polar lipids in the fish are given in Table 3. The effect of feeding time on these characteristics is given in the form of equations in Table 4. The total lipid level in the fish was affected

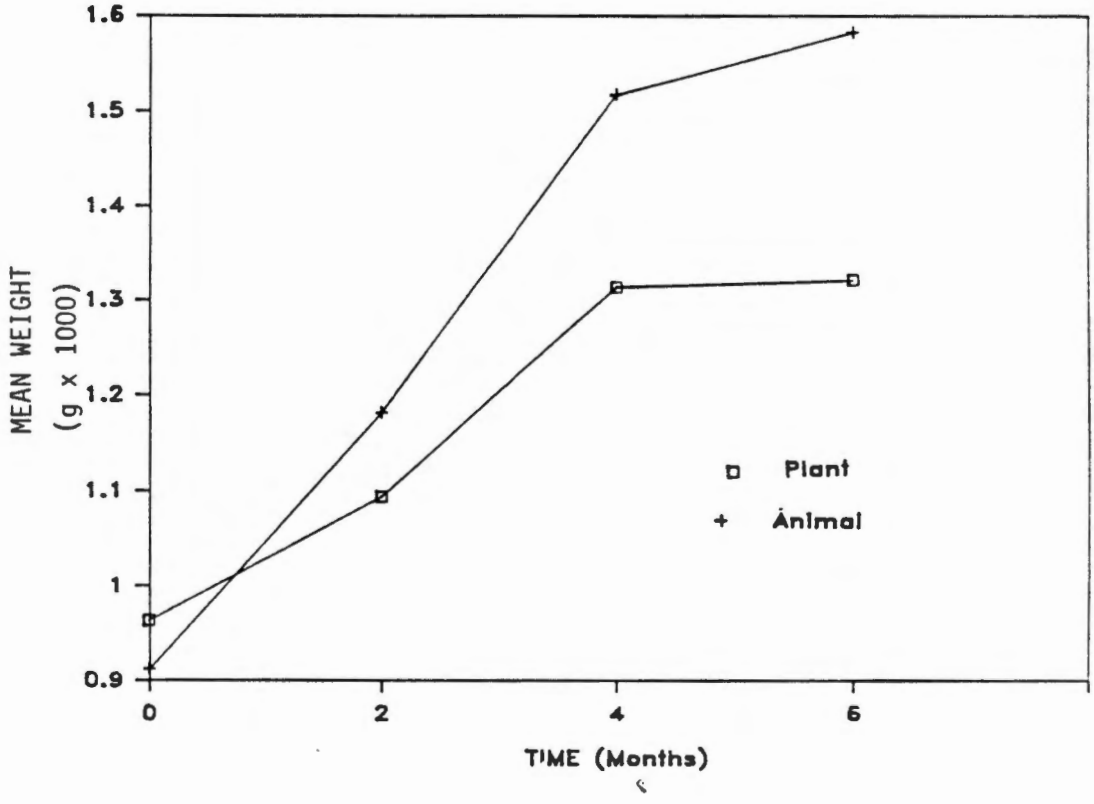

Figure 1. Mean Weight Gain of Grass Carp Fed Different Diets Up to 6 Months.

Table 3. Content of Total Lipid and Percentage Phospholipid Classes in White Amur Fed Animal and Plant Diets from 0 to 6 Months.

Lipid	Diet[a]	Months 0	2	4	6
		----------% of Fish Meat----------			
Total	Animal[b]	1.80	2.18	3.72	2.28
	Plant	1.80	0.98	0.81	0.73
		------% of Total Phospholipids--------			
Sphingomyelin	Animal[b]	10.64	9.85	7.11	6.45
	Plant[b]	10.64	7.93	13.54	8.07
Lecithin	Across[b]	62.71	60.59	54.79	61.98
Cephalin	Across[b]	26.65	30.53	34.88	30.76

[a]For each lipid class if diet listed as plant and animal, a diet by feeding time interaction was found ($P < 0.05$); if listed as across, no significant interaction was found and percentage for each time was averaged across diets.

[b]For each diet or across diet a significant feeding time effect was found.

Table 4. Equations Showing Significant Feeding Time Effects on Levels of Total Lipid and Percentage Phospholipid Classes in White Amur Fed Animal and Plant Diets 0 to 6 Months.

Lipid Class	Diet[a]	R^2	Equation[b]
Total lipid	Animal	0.156	$Y = 1.80 - 0.782T + 0.657T^2 - 0.085T^3$
	Plant	NS[c]	
Sphingomyelin	Animal	0.067	$Y = 10.21 - 0.784T$
	Plant	0.152	$Y = 10.64 - 6.67T + 3.46T^2 - 0.045T^3$
Lecithin	Across	0.089	$Y = 64.30 - 4.306T + 0.631T^2$
Cephalin	Across	0.122	$Y = 25.80 + 4.054T - 0.526T^2$

[a]Animal - trout chow pellets; Plant = alfalfa pellets; Across = insignificant treatment by feeding time interaction.

[b]Y = % of total lipid in meat or % phospholipid class in total phospholipid depending upon lipid class. T = feeding time in months; all were significant at the $P < 0.05$ level.

[c]NS = not significant.

by treatment (diet) and feeding time ($P<0.05$), and had a significant treatment x feeding time interaction. The total lipid level in fish fed trout chow increased from 1.80% at the beginning of the feeding period to 3.72% at the end of 4 months before decreasing to 2.28% ($P< 0.05$). The total lipid level in fish fed alfalfa pellets was not affected significantly by feeding time even though there appeared to be a decrease in total lipid level in fish after being fed alfalfa pellets.

The level of sphingomyelin in the fish polar lipids was affected significantly by feeding time, and the interaction of treatment by feeding time but not by treatment (diet). Expressed as a percentage of total phospholipids, sphingomyelin content in fish fed the trout chow decreased linearly from 10.64% at the beginning of the feeding experiment to 6.45% after 6 months. In fish fed the alfalfa pellets, however, there was a cubic relationship between percentage sphingomyelin and feeding time. Fish fed the plant diet for 4 months had the highest percentage sphingomyelin while fish fed for 2 months had the lowest level (Tables 3 and 4). No differences between diets ($P<0.05$) or significant diet x feeding time interactions were found for the percentages of lecithin and cephalin in fish polar lipids. As the feeding time increased from 0 to 4 months, the percentage lecithin decreased from 62.7 to 54.79% before increasing to approximately 62% at 6 months feeding time. The inverse of this trend was found for the percentage of cephalin. The levels of cephalin increased from 26.265%

at 0 month to 34.88% at 4 months feeding time before decreasing to 30.76% at 6 months (Table 3).

Measurement of each phospholipid, however, at 205 nm was dependent upon the number of double bonds in its fatty acids (Chen & Kou, 1982). Therefore, changes in percentages of each phospholipid across feeding time is confounded with the change in its unsaturated fatty acid composition.

The fact that phosphatidyl choline and phosphatidyl ethanolamine were the most abundant phospholipids are in agreement with several researchers who have studied phospholipid composition in aquatic invertebrates and in the muscles of fishes (Addison et al., 1968, 1972; Cossins, 1976; deKoning & McMullan, 1966; deKoning, 1966a, b; Gray & MacFarlane, 1961; Nair & Gopakumar, 1984; Patton, 1975). These same investigators also found small levels of sphingomyelin in these tissues as was found in the present study. No reports of studies were found, however, in which the phospholipids in grass carp had been investigated.

Grass carp are considered as a low-oil content fish since the range of low oil reported in other fish varies from 0.1 to 5.0% as reported by Stansby (1984). In the present study, the fish fed the animal diet (3.72%) were in the middle to upper part of this range, and fish fed the plant diet (2.73%) were in the lower part of the range (Table 3).

Fatty Acid Content of the Fish Phospholipids

The percentages of fatty acids in fish phospholipids averaged across feeding time for each diet are shown in Table 5. Nineteen fatty acids were identified in the phospholipids of the fish fillet: 14:0 (myristic), 16:0I (isopalmitic), 16:0 (palmitic), 16:1 (palmotoleic), 18:0 (stearic), 18:1 (oleic), 18:0I (isooleic), 18:2ω6 (linoleic), 18:3ω3 (linolenic), 18:3ω6 (isolinolenic), 20:1 (eicosenoic), 20:2 (eicosadienoic), 20:3 (eicosatrienoic), 20:4ω6 (arachidonic), 20:5ω3 and 20:5ω6 (eicosapentenoic acids), 22:5ω3 (docosapentaenoic), 22:6ω3 (docosahexaenoic) and 24:1 (tetracosenoic). Differences were found between diet treatments at the $P < 0.05$ level for all fatty acids except 16:0I, 18:0, 18:1 and 20:1. Fish fed the animal diet had higher percentages of 14:0, 16:0, 16:1, 18:11, 18:3ω 6, 20:5ω3, 22:5ω3, and 22:6ω3 in their phospholipids than did fish fed the plant diet. Compared with fish fed the animal diet, fish fed the alfalfa pellets (plant diet) had higher percentages of 18:2ω6, 18:3ω3, 20:2, 20:3, 20:4ω6, 20:5ω6 and 24:1 in their phospholipids. The most abundant fatty acids found in the fish's phospholipids were 16:0, 22:6ω3, 20:4ω6, 18:1, and 18:0 as seen in Table 5.

Percentages of fatty acids in the phospholipids of the White Amur at 0, 2, 4, and 6 months feeding time are given in Table 6, and equations showing significant feeding time effects for the various fatty acids are given in Table 7. Feeding time significantly affected the percentages of 16:0I, 16:0, 16:1, 18:1, 18:1I. 18:2ω6, 18:3ω6, 18:3ω3, 20:1, 20:2, 20:3, 20:4ω6, 20:5ω3, 22:6ω3 and 24:1.

Table 5. Percentage of Fatty Acids in Phospholipids of White Amur Averaged Across Feeding Times for Each Diet.

Fatty Acid	Diet	
	Animal[a]	Plant[a]
14:0	0.98[b]	0.65[c]
16:0I	1.97	2.70
16:0	24.57[b]	21.70[c]
16:1	1.87[b]	1.41[c]
18:0	7.37	7.03
18:1	8.06	7.34
18:1I	2.96[b]	2.24[c]
18:2ω6	6.35[b]	9.45[c]
18:3ω3	0.41[b]	2.40[c]
18:3ω6	0.05[b]	0.01[c]
20:1	0.27	0.22
20:3	0.56[b]	0.89[c]
20:4ω6	11.13[b]	16.54[c]
20:5ω3	9.23[b]	4.62[c]
20:5ω6	0.63[b]	0.84[c]
22:5ω3	2.17[b]	1.92[c]
22:6ω3	18.57[b]	15.56[c]
24:1	1.77[b]	2.73[c]

[a]Animal = trout chow pellets; Plant = alfalfa pellets.

[b,c]Means in a row followed by superscripts are significantly different at the $P < 0.05$ level.

Table 6. Average Percentage Fatty Acids in Phospholipids of White Amur Fed Plant and Animal Diets for 0 to 6 Months.

Acid	Diet[a]	Feeding Time in Months			
		0	2	4	6
14:0	Across	0.91	0.99	0.66	0.70
16:0I	Across[b]	4.53	1.92	2.71	1.33
16:0	Animal[b]	24.47	23.68	23.68	26.25
	Plant[b]	24.47	21.73	19.27	22.71
16:1	Across[b]	1.11	1.68	1.83	1.68
18:0	Across	7.06	7.49	7.29	6.90
18:1	Across[b]	8.94	8.23	7.30	6.88
18:1I	Animal	2.84	3.02	3.15	2.81
	Plant[b]	2.84	2.48	1.92	2.01
18:2ω6	Animal[b]	9.14	7.56	5.36	4.56
	Plant	9.14	9.55	8.53	10.44
18:3ω3	Animal	0.70	0.65	0.20	0.22
	Plant[b]	0.70	1.10	3.07	3.87
18:3ω6	Animal[b]	0.00	0.04	0.17	0.00
	Plant	0.00	0.00	0.02	0.00
20:1	Across	0.16	0.21	0.29	0.27
20:2	Animal[b]	0.89	0.64	0.51	0.35
	Plant	0.89	0.82	0.96	0.88
20:3	Animal[b]	1.40	1.25	0.88	0.84
	Plant[b]	1.40	1.13	2.18	2.11
20:4ω6	Animal[b]	13.97	12.76	9.66	9.28
	Plant[b]	13.97	14.74	17.72	18.43
20:5ω6	Across	0.60	0.71	0.88	0.70
20:5ω3	Animal[b]	3.43	6.17	11.07	13.66
	Plant	3.43	5.40	4.64	4.44
22:5ω3	Across	1.95	2.05	2.23	1.92
22:6ω3	Animal[b]	15.44	18.75	19.99	18.76
	Plant[b]	15.44	17.37	15.91	13.45
24:1	Animal[b]	2.45	2.11	1.72	1.14
	Plant	2.45	2.44	2.83	3.00

[a]Animal = trout chow pellets; Plant = alfalfa pellets; Across = insignificant treatment by feeding time interaction.

[b]Significantly affected by feeding time ($P < 0.05$).

Table 7. Equations Showing the Significant Effects of Feeding Time on the Percentages of the Fatty Acids in the Phospholipids of the White Amur.

Fatty Acid	Diet[a]	R^2	Equation[b]
14:0	Across	.NS[c]	
16:0I	Across	0.302	$Y = 4.54 - 3.08T + 1.12T^2 - 0.116T^3$
16:0	Animal	0.088	$Y = 24.61 - 1.012T + 0.212T^2$
	Plant	0.197	$Y = 24.00 - 2.846T + 0.403T^2$
18:0	Across	.NS	
16:1	Across	0.138	$Y = 1.12 + 0.365T - 0.406T^2$
18:1	Across	0.267	$Y = 8.89 - 0.351T$
18:1I	Animal	.NS	
	Plant	0.117	$Y = 2.73 - 0.145T$
18:2ω6	Animal	0.168	$Y = 9.01 - 0.783T$
	Plant	.NS	
18:3ω6	Animal	0.323	$Y = 0.062T + 0.057T^2 - 0.008T^3$
	Plant	.NS	
18:3ω3	Animal	.NS	
	Plant	0.342	$Y = 0.33 + 0.602T$
20:1	Across	.NS	
20:2	Animal	0.195	$Y = 0.84 - 0.083T$
	Plant	.NS	
20:3	Animal	0.061	$Y = 1.40 - 0.102T$
	Plant	0.301	$Y = 1.40 - 0.866T + 0.467T^2 - 0.050T^3$
20:4ω6	Animal	0.084	$Y = 14.03 - 0.854T$
	Plant	0.083	$Y = 13.65 + 0.843T$
20:5ω3	Animal	0.469	$Y = 3.24 + 1.795T$
	Plant	.NS	
20:5ω6	Across	.NS	
22:5ω3	Across	.NS	
22:6ω3	Animal	0.123	$Y = 15.40 + 2.271T - 0.285T^2$
	Plant	0.140	$Y = 15.66 + 1.218T - 0.268T^2$
24:1	Animal	0.140	$Y = 2.53 - 0.222T$
	Plant	.NS	

[a]Animal = Trout chow pellets; Plant = Alfalfa pellets; Across = insignificant treatment by feeding time interaction.

[b]Y = percentage of fatty acid and T = feeding time in months.

[c]NS = not significant.

Significant treatment x feeding time interactions were found for 16:0, 18:1I, 18:2ω6, 18:3ω6, 18:3ω3, 20:2, 20:3, 20:4ω6, 20:5ω3, 22:6ω3 and 24:1.

The percentage of 16:0I averaged across diets had a cubic relationship with feeding time; it decreased from 4.53 at 0 months to 1.92 at 2 months and then increased to 2.71 at 4 months before decreasing to 1.33 at 6 months (Tables 6 and 7). The percentage of 16:1 averaged across diets increased quadratically from 1.11 at 0 months to 1.83 at 4 months before decreasing again. The percentage of 18:1 averaged across diets decreased linearly with increasing feeding time from 8.94 at 0 months to 6.88 at 6 months.

Several of the fatty acids in the phospholipids for which a significant interaction was found changed significantly ($P < 0.05$) in percentage across feeding time in fish fed one diet but did not change in fish fed the other diet. The percentages of the following fatty acids changed significantly across feeding time in fish fed the animal diet but not in fish fed the plant diet: 18:2ω6, 18:3ω6, 20:2, 20:5ω3 and 24:1. The percentages of 18:1I and 18:3ω3 changed significantly across feeding time in fish fed alfalfa pellets but not in fish fed trout chow. The percentages of 18:2ω6, 20:2, and 24:1 decreased linearly across feeding time in fish fed trout chow while the percentage of 20:5ω3 increased linearly (Tables 6 and 7). The percentage of 18:3ω6 in phospholipids of fish fed trout chow had a cubic relationship with feeding time (Table 7). In the phospholipids

of fish fed alfalfa pellets, the percentage of 18:1I decreased linearly across feeding time, and the percentage of 18:3ω3 increased linearly (Tables 6 and 7).

Percentages of 16:0, 20:3, 20:4ω6, and 22:6ω3 each changed in a different manner across feeding time in the phospholipids of fish fed trout chow compared with fish fed alfalfa pellets. In fish fed trout chow the percentage of 16:0 decreased from 24.47 at 0 months to 23.68 at 4 months before increasing to 26.25 at 6 months feeding time, but in fish fed alfalfa pellets, the percentage 16:0 decreased from 24.47 at 0 months to 19.27 at 4 months before increasing to 22.7 at 6 months feeding time (Table 6). The percentage of 20:3 decreased linearly from 1.40 at 0 months to 0.84 at 6 months in fish fed trout chow, but in fish fed alfalfa pellets, the percentage 20:3 had a cubic relationshp with feeding time (Tables 6 and 7). The percentage of 20:4ω6 decreased linearly from 13.97 at 0 months to 9.29 at 6 months in fish fed trout chow. In contrast to this change, the percentage of 20:4ω6 in fish fed alfalfa pellets increased linearly from 13.97 at 0 months to 18:44 at 6 months feeding time. In fish fed trout chow, the percentage of 22:6ω3 in the phospholipids increased to a maximum at approximately 4 months feeding time before decreasing, but the pecentage of this acid in the phospholipids of fish fed alfalfa pellets increased to a maximum at 2 months feeding time before decreasing curvilinearly with increasing feeding time up to 6 months (Tables 6 and 7).

The percentages of fatty acid types in the phospholipids of white amur fed different diets averaged across feeding times are given in Table 8. Fish fed the animal diet overall contained more saturated, monounsaturated and Omega-3 fatty acid types and less omega-6 fatty acids than did fish fed the plant diet.

The percentages of fatty acid types in the phospholipids of white amur for each feeding time are shown in Table 9, and the equations showing significant feeding time effects on the fatty acid types in Table 10. In fish fed the animal diet, the percentage saturated fatty acids decreased quadratically to a minimum level between 2 and 3 months before increasing to 35.98 at 6 months feeding time. In contrast to this trend, the percentage saturated fatty acids in phospholipids of the fish fed the plant diet decreased to a minimum level between 4 and 6 months. The percentage monounsaturated fatty acids in the phospholipids of the white amur fed both diets decreased linearly from 15.50 at 0 month feeding time to 13.35 at 6 months (Tables 9 and 10). The percentage of Omega-6 fatty acids in the phospholipids of the fish fed the animal diet decreased linearly from 26.01 at 0 months to 15.54 at 6 months feeding time in contrast to the linear increase in the percentage of these acids in fish fed the plant diet from 16.01 at 0 months to 32.77 at 6 months (Tables 9 and 10). The percentage Omega-3 fatty acids increased linearly from 21.52 at 0 months to 34.73 at 6 months feeding time in the phospholipids of the fish fed the animal diet and increased

Table 8. Percentage Fatty Acid Types in the Phospholipids of White Amur Averaged Across Feeding Times for Each Diet.

Fatty acid type[a]	Diet[b]	
	Animal	Plant
Saturated	34.89[c]	32.08[d]
Monounsaturated	14.94[c]	13.93[d]
Omega-6	19.77[c]	29.48[d]
Omega-3	30.39[c]	24.51[d]

[a]Saturated includes 14:0. 16:0, 16:0I, 17:0, and 18:0; Monounsaturated includes 16:1, 17:1, 18:1, 18:1I, 20:1, and 24:1; Omega-6 includes 18:2ω6, 218:3ω6, 20:2, 20:3, 20:4ω6, and 20:5ω6; and Omega-3 includes 18:3ω3, 20:5ω3, 22:5ω3, and 22:6ω3.

[b]Animal = trout chow pellets and plant = alfalfa pellets.

[cd]Means in a row bearing unlike superscripts are significantly different at the $P<0.05$ level.

Table 9. Average Percentage Fatty Acid Types in Phospholipids of White Amur Fed Plant and Animal Diets for 0 to 6 Months.

Fatty acid type[a]	Diet[b]	Feeding time in months			
		0	2	4	6
Saturated	Animal[c]	36.97	33.63	33.87	35.98
	Plant[c]	36.97	32.58	30.42	30.77
Monounsaturated	Across[c]	15.50	15.15	14.23	13.35
Omega-6	Animal[c]	26.01	22.97	17.28	15.54
	Plant[c]	16.01	26.97	30.45	32.77
Omega-3	Animal[c]	21.52	27.77	33.64	34.73
	Plant[c]	21.52	25.77	25.74	32.51

[a]Saturated = 14:0, 16:0, 16:0I, 17:0, and 18:0; Monounsaturated = 16:1, 17:1, 18:1, 18:1I, 20:1, and 24:1; Omega-6 = 18:2ω6, 18:3ω6, 20:2, 20:3, 20:4ω6, and 20:5ω6; Omega-3 = 18:3ω3, 20:5ω3, 22:5ω3, and 22:6ω3.

[b]Animal = trout chow pellets; Plant = alfalfa pellets; Across = insignificant treatment by feeding time interaction.

[c]Significantly affected by feeding time ($P < 0.05$).

Table 10. Equations Showing the Significant Effects of Feeding Time on the Percentages of the Fatty Acid Types in the Phospholipids of White Amur.

Fatty acid Type[a]	Diet[b]	R^2	Equation[c]
Saturated	Animal	0.083	$Y = 36.82 - 2.165T + 0.340T^2$
	Plant	0.244	$Y = 37.00 - 2.826T + 0.298T^2$
Monounsaturated	Across	0.333	$Y = 15.75 - 0.388T$
Omega-6	Animal	0.164	$Y = 26.05 - 1.846T$
	Plant	0.075	$Y = 25.02 + 1.248T$
Omega-3	Animal	0.333	$Y = 23.12 + 2.139T$
	Plant	0.041	$Y = 21.71 + 2.674T - 0.398T^2$

[a]See Table 9.

[b]Animal = trout chow pellets; Plant = alfalfa pellets; Across = insignificant treatment by feeding time interaction.

[c]Y = Percentage fatty acid type and T = feeding time in months.

quadratically in the phospholipids of the fish fed the plant diet (Tables 9 and 10).

Fatty Acids in Neutral Lipids of White Amur

Percentages of fatty acids in the neutral lipids of the fish averaged across feeding times for each treatment (diet) are shown in Table 11. Twenty-seven fatty acids were found in the neutral lipids. Nine of these fatty acids were not found in the phospholipids of the fish (Table 5): 16:1I (isopalmitoleic), 17:0 (heptadecanoic), 17:1 (heptadecenoic), 20:0 (arachidic), 22:1 (docosenoic), 23:0 (tricosanoic), 24:0 (tetracosanoic), 24:4 (tetracosatetraenoic), and an unknown. Differences between treatments ($P<0.05$) were found for 14:0, 16:0, 16:1, 17:0, 17:1, 18:0, 18:1, 18:3ω3, 20:1, 20:2, 20:3, 20:4ω6, 23:0, 24:0, 24:1, 24:4, and the unknown. Fish fed the animal diet (trout chow) had higher percentages of 14:0, 16:1, 17:1, 18:1, 20:1, 22:1, 23:0, 24:0, 24:4, and unknown acid and lower percentages of 16:0, 17:0, 18:0, 18:3ω3, 20:2, 20:3, 20:4ω6, and 24:1 in their neutral lipids than did fish fed the plant diet (alfalfa pellets).

Percentages of each fatty acid in the neutral lipids of the white amur for 0, 2, 4, and 6 months feeding times are shown in Table 12, and the equations showing the significant feeding time effects are given in Table 13. The percentages of the following fatty acids: 14:0, 16:0, 16:1, 16:1I, 17:0, 17:1, 18:2ω6, 18:3ω3, 20:0, 20:1, 20:2, 20:3, 20:4ω6, 22:1, 23:0, 24:0, 24:1, 24:4, and the unknown acids were significantly affected by feeding time. A significant

Table 11. Percentage Fatty Acids in Neutral Lipids of White Amur Averaged Across Feeding Times for Each Diet.

Fatty Acid	Diet	
	Animal[a]	Plant[a]
14:0	5.03[b]	1.84[c]
16:0	21.51[c]	23.75[b]
16:1	9.97[b]	3.45[c]
16:1I[d]	0.26	0.13
17:0[d]	0.73[c]	2.30[b]
17:1[d]	0.88[b]	0.45[c]
18:0	4.61[c]	7.78[b]
18:1	22.10[b]	13.42[c]
18:1I	0.99	1.88
18:2ω6	13.21	14.66
18:3ω6	0.15	0.19
18:3ω3	1.58[c]	5.12[b]
20:0[d]	0.35	0.27
20:1	1.15[b]	0.59[c]
20:2[d]	0.50[c]	0.82[b]
20:3	0.66[c]	1.45[b]
20:4ω6	2.09[c]	9.15[b]
20:5ω3	4.34	3.53
22:1	0.81[b]	0.48[c]
22:5ω3	1.94	1.51
22:5ω6	0.55	0.68
22:6ω3	5.04	5.24
23:0[d]	0.36[b]	0.00[c]
24:0[d]	0.40[b]	0.00[c]
24:1[d]	0.44[c]	1.30[b]
24:4[d]	0.25	0.00[c]
Unknown	0.10[b]	0.00[c]

[a]Animal = trout chow pellets; Plant = alfalfa pellets.

[b,c]Means in a row bearing unlike superscripts are significantly different at the $P<0.05$ level.

[d]Tentatively identified.

Table 12. Mean Percentage of Fatty Acids in Neutral Lipid of White Amur Fed Animal and Plant Diets from 0 to 6 Months.

Fatty Acid	Diet[a]	Time in Months 0	2	4	6
		----------%			
14:0	Animal[a]	2.04	5.05	5.62	5.88
	Plant	2.04	1.98	1.88	1.51
16:0	Animal[a]	21.94	22.88	21.29	20.14
	Plant[a]	21.94	22.30	25.69	24.22
16:1	Animal[a]	4.26	8.79	11.33	12.65
	Plant	4.26	3.38	3.56	2.91
16:1I[c]	Across[a]	0.34	0.40	0.10	0.00
17:0[c]	Animal	0.96	0.74	0.59	0.73
	Plant[a]	0.96	1.43	3.06	3.23
17:1[c]	Animal[a]	0.46	0.55	1.01	1.30
	Plant	0.46	0.43	0.43	0.48
18:0	Across	6.41	5.99	5.36	7.07
18:1	Across	20.62	18.15	17.45	16.51
18:2ω6	Across[a]	17.46	15.80	12.16	11.87
18:1I	Across	1.43	1.01	2.43	0.76
18:3ω6	Across	0.19	0.16	0.17	0.15
18:3ω3	Animal	1.81	1.67	1.20	1.79
	Plant[a]	1.81	2.98	7.05	7.35
20:0[c]	Animal[a]	0.00	0.04	0.69	0.52
	Plant	0.00	0.50	0.23	0.20
20:1	Animal[a]	0.77	1.12	1.24	1.26
	Plant[a]	0.77	0.75	0.45	0.44
20:2	Animal[a]	0.80	0.56	0.42	0.38
	Plant	0.80	0.93	0.77	0.77
20:3	Animal[a]	1.51	0.69	0.40	0.45
	Plant	1.51	1.36	1.44	1.50
20:4ω6	Animal[a]	6.94	1.62	1.09	1.13
	Plant	6.94	10.15	8.03	10.61
20:5ω3	Across	3.63	4.21	3.95	3.83
22:1	Across[a]	0.29	0.64	0.72	0.78
22:5ω6	Across	0.73	0.61	0.57	0.61
22:5ω3	Across	1.68	1.71	1.55	2.04
22:6ω3	Across	4.51	5.01	5.32	5.42
23:0	Animal[a]	0.00	0.36	0.38	0.51
	Plant	0.00	0.00	0.00	0.00
24:0	Animal[a]	0.00	0.40	0.51	0.50
	Plant	0.00	0.00	0.00	0.00
24:1	Animal[a]	1.21	0.44	0.24	0.24
	Plant	1.21	1.21	1.14	1.65
24:4	Animal[a]	0.00	0.28	0.33	0.24
	Plant	0.00	0.00	0.00	0.00
Unknown	Animal[a]	0.00	0.00	0.19	0.16
	Plant	0.00	0.00	0.00	0.00

[a]Significantly affected by feeding time.

[b]Tentatively identified.

Table 13. Equations Showing Significant Effects of Feeding Time of Different Diets on Fatty Acids in Neutral Lipid of White Amur.

Fatty Acid	Diet[a]	R^2	Equations[b]
14:0	Animal	.193	$y = 2.24 + 1.581T - 0.163T^2$
	Plant	NS[c]	
16:0	Animal	.079	$y = 22.97 - 0.426T$
	Plant	.225	$y = 21.94 - 1.883T + 1.362T^2 - 1.64T^3$
16:1	Animal	.237	$y = 4.33 + 2.570T - 0.198T^2$
	Plant	NS	
16:1I	Across	.151	$y = 0.44 - 0.514T$
17:0	Animal	NS	
	Plant	.202	$y = 0.86 + 0.434T$
17:1	Animal	.343	$y = 0.34 + 0.1587$
	Plant	NS	
18:0	Across	NS	
18:1	Across	NS	
18:1I	Across	NS	
18:2	Across	.415	$y = 17.36 - 1.021T$
18:3ω6	Across	NS	
18:3ω3	Animal	NS	
	Plant	.223	$y = 1.60 + 1.066T$
20:0	Animal	.328	$y = -0.114T + 0.074T^2 - 0.0084T^3$
	Plant	NS	
20:1	Animal	.091	$y = 0.78 + 0.203T - 0.21T^2$
	Plant	.063	$y = 0.81 - 0.069T$
20:2	Animal	.145	$y = 0.72 - 0.063T$
	Plant	NS	
20:3	Animal	.195	$y = 1.50 - 0.498T + 0.054T^2$
	Plant	NS	
20:4ω6	Animal	.045	$y = 4.56 - 0.72T$
	Plant	NS	
20:5ω3	Across	NS	
22:1	Across	.192	$y = 0.42 + 0.69T$
22:5ω6	Across	NS	
22:5ω3	Across	NS	
22:6ω3	Across	NS	
23:0	Animal	.239	$y = 0.336T - 0.097T^2 + 0.0092T^3$
	Plant	NS	
24:0	Animal	.230	$y = 0.02 + 0.227T - 0.0245T^2$
	Plant	NS	
24:1	Animal	.133	$y = 1.17 - 0.432T + 0.047T^2$
	Plant	NS	
24:4	Animal	.240	$y = 0.01 + 0.16$
	Plant	NS	
Unknown	Animal	.306	$y = 0.114T + 0.074T^2 - 0.0084T^3$
	Plant	NS	

[a]Animal = trout chow pellets; Plant = alfalfa pellets; Across = insignificant treatment by feeding time interactions.

[b]y = percentage of fatty acid and T = feeding time in months.

[c]NS = not significant.

treatment x feeding time effect was found for all of these acids except 16:1I, 18:2ω6, and 22:1. Percentages of 14:0, 16:1, 17:1, 20:0, 20:2, 20:3, 20:4ω6, 23:0, 24:0, 24:1, 24:4, and the unknown changed significantly across feeding time in the neutral lipids of fish fed the animal diet but not the plant diet (Table 13). Percentages of 17:0 and 18:3ω3 were significantly affected by feeding time in fish fed the plant diet but not the animal diet. Percentages of 16:0 and 20:1 changed across feeding time in the neutral lipids of fish fed either diet, but the pattern of the change for each acid differed between the diets (Table 13).

In the neutral lipids of fish fed the animal diet, percentages of 16:0, 20:2 and 20:4ω6 decreased linearly across feeding time, and percentages of 17:1, and 24:4 increased linearly with increasing feeding time (Table 13). With increasing feeding time, the percentages of 14:0, 16:1, 20:1, and 24:1 increased quadratically to a maximum between 4 and 5 months feeding time while percentages of 20:3 and 24:1 decreased quadratically to a minimum. Also, in fish fed the animal diet, a cubic relationshp was found between the percentage of each of the following neutral lipid fatty acids: 20:0, 23:0 and the unknown, and the feeding time (Table 13).

In the neutral lipids of the fish fed the plant diet, percentages of 17:0 and 18:3ω3 increased linearly across feeding time while the percentage of 20:1 decreased linearly (Tables 12 and 13). In addition, the percentage of 16:0 had a cubic relationship with

feeding time and was higher in fish fed the plant diet for 2 to 6 months than in fish at 0 months (Tables 12 and 13).

In the neutral lipids of the fish fed either diet, the pecentages of 16:1I and 22:1 increased linearly across feeding time, and the percentage of 18:2ω6 decreased linearly (Tables 12 and 13).

The percentage fatty acids types in the neutral lipids of white amur averaged across feeding times for each diet are given in Table 14. Fish fed the animal diet contained a higher percentage of monounsaturated fatty acids but lower percentages of saturated, Omega-6 and Omega-3 types of fatty acids than did fish fed the plant diet.

The percentages fatty acid types in the neutral lipids of white amur fed different diets for 0 to 6 months are given in Table 15, and the equations showing the significant feeding time effects on these fatty acid types are shown in Table 16. Feeding time had no significant effect on the percentage of saturated fatty acid types in the neutral lipids of fish fed the animal diet; however, in fish fed the plant diet, the percentage of saturated fatty acid increased linearly across feeding time (Tables 14 and 15). Feeding time had no significant effect on the percentage monounsaturated fatty acids in the neutral lipids of fish fed either diet (Table 15). The percentage of Omega-6 fatty acids decreased quadratically in the neutral lipids of fish fed the animal diet from 27.64 at 0 months to a minimum level of approximately 14.20 between 4 and 6 months feeding time (Tables 14 and 15). In contrast to the relationship of the percentage of Omega-6

Table 14. Percentage Fatty Acid Types in Neutral Lipids of White Amur Averaged Across Feeding Time for Each Diet.

Fatty acid type[a]	Diet[b]	
	Animal	Plant
Saturated	32.98[c]	35.94[d]
Monounsaturated	36.59[d]	21.70[c]
Omega-6	17.41[c]	26.94[d]
Omega-3	12.91[c]	15.42[d]

[a]Saturated includes 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 23:0, and 24:0; Monounsaturated includes 16:1, 16:1I, 17:1, 18:1, 18:1I, 20:1, 22:1, and 24:1; Omega-6 includes 18:2ω6, 18:3ω6, 20:4ω6, 20:5ω6, 20:2, 20:3, and 24:4; and the Omega-3 includes 18:3ω3, 20:5ω3, and 22:6ω3.

[b]Animal = trout chow pellets and Plant = alfalfa pellets.

[cd]Means in a row bearing unlike superscripts are significantly different at the $P<0.05$ level.

Table 15. Average Percentage Fatty Acid Types in Neutral Lipids of White Amur Fed Plant and Animal Diets 0 to 6 Months.

Fatty acid type[a]	Diet[b]	Feeding time in months			
		0	2	4	6
Saturated	Animal	31.36	33.69	32.22	33.84
	Plant[c]	31.36	33.98	38.43	38.05
Monounsaturated	Across	29.38	28.54	30.41	28.98
Omega-6	Animal[c]	27.64	18.51	14.28	14.34
	Plant[c]	27.64	30.24	23.98	26.13
Omega-3	Across	11.63	13.26	15.04	15.49

[a]Saturated includes 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 23:0, and 24:0; Monounsaturated includes 16:1, 16:1I, 17:1, 18:1, 18:1I, 20:1, 22:1, and 24:1; Omega-6 includes 18:2ω6, 18:3ω6, 20:4ω6, 22:5ω6, 20:2, 20:3, and 24:4; and Omega-3 includes 18:3ω3, 20:5ω3, 22:5ω3, and 22:6ω3.

[b]Animal = trout chow pellets; Plant = alfalfa pellets; Across = insignificant treatment by feeding time interaction.

[c]Significantly affected by feeding time ($P < 0.05$).

Table 16. Equations Showing the Significant Effects of Feeding Time On the Percentages of Fatty Acid Types in the Neutral Lipids of White Amur.

Fatty acid Type[a]	Diet[b]	R^2	Equation[c]
Saturated	Animal	NS[d]	
	Plant	0.150	$Y = 31.94 + 1.210T$
Monoun-saturated	Across	NS	
Omega-6	Animal	0.238	$Y = 27.58 - 5.630T + 0.571T^2$
	Plant	0.067	$Y = 27.58 + 6.397T - 3.268T^2 + 0.360T^3$
Omega-3	Across	0.091	$Y = 11.96 + 0.648T$

[a]See Table 15.

[b]Animal = trout chow pellets; Plant = alfalfa pellets; Across = insignificant treatment by feeding time interaction.

[c]Y = Percentage fatty acid type and T = feeding time in months.

[d]NS = not significant.

fatty acids with feeding time in fish fed the animal diet, the percentage of Omega-6 fatty acids in fish fed the plant diet had a cubic relatinship with feeding time as shown in the same tables. The percentage of Omega-3 fatty acids in the neutral lipids of fish fed both diets increased linearly from 11.63 at 0 months feeding time to 15.49 at 6 months (Table 15).

Fatty Acid Composition of Total Lipids in the Fish Diets

The percentage of total lipids in the animal diet was 12.60 and in the plant diet, 3.36. The percentages of fatty acids in the total lipids of each fish diet are shown in Table 17. The animal diet had twenty-one fatty acids compared to only thirteen fatty acids found in the alfalfa pellets. The fatty acids in the animal diet or trout chow not present in the alfalfa pellets such as 15:0, 16:1, 17:0, 20:0, 20:1, 20:3, 22:0, 22:1, 22:5ω3, 22:6ω3, 24:0, and 24:1. In contrast to the fatty acids present in the animal diet, the following fatty acids: 12:0, 13:0, 14:0I and 16:0I, were present in the plant diet. There were also differences in the most abundant fatty acids between the diets. The most abundant fatty acids only in the animal diet were 16:0, 18:1, 18:2ω6, 20:5ω3, and 16:1, and the most abundant in the plant diet were 16:0, 18:3ω3, and 18:2ω6.

Diet Lipid Level and Fish Growth

The higher level of total lipids in the animal versus the plant diet (12.6 vs 3.36%) is at least partially responsible for the faster weight gain and higher lipids content in the fish fed the

Table 17. Percentage Fatty Acids in the Total Lipids of Each Fish Diet.

Fatty Acid	Diet	
	Animal[a]	Plant[a]
12:0	-	1.62
13:0	-	0.74
14:0	7.64	4.18
14:01	-	1.47
15:0	0.52	-
16:01	-	7.16
16:0	24.08	30.31
16:1	9.56	-
17:0	0.98	-
18:0	6.41	4.77
18:1	16.13	4.76
18:11	3.40	0.60
18:2ω6	11.18	17.40
18:3ω3	0.96	24.66
20:0	0.96	.82
20:1	1.35	-
20:3	0.18	-
20:4ω6	0.95	1.51
20:5ω3	10.53	-
22:0	0.25	-
22:1	0.20	-
22:5ω3	0.91	-
22:6ω3	3.40	-
24:0	0.06	-
24:1	0.33	-

[a]Animal = trout chow pellets; Plant = alfalfa pellets.

animal diet compared with the fish fed the plant diet. However, the trout pellets are a complete fish ration with the correct levels of protein, vitamin and minerals (Bakir, 1985), and the alfalfa pellets are not a complete ration. Therefore, the fish would be expected to grow faster and deposit more fat if fed the animal diet rather than the plant diet.

Relationships Between Fish Lipid Fatty Acids and Dietary Fatty Acids

In general, the differences found between the diets for the percentages of polyunsaturated fatty acids in the fish phospholipids were due to the fatty acid compositions of the diets. The higher percentages of 18:2ω6, 18:3ω3, and 20:4ω6 in the fish fed the plant diets compared with their respective levels in fish fed animal diets (Table 5, p. 41) are most likely due to the higher levels of these acids in the total lipids of the plant diet versus the animal diets (Table 5, p. 51). Also, the higher levels of 20:5ω3 and 22:6ω3 in the animal diet compared with the plant diet (Table 11, p. 51) are most likely the reason for the higher levels of these acids in the phospholipids of the fish fed the animal compared to those fed the plant diet (Table 5, p. 41).

Differences between diets in the saturated and monounsaturated fatty acid levels in the total lipids (Table 11, p. 51) were better reflected in the neutral lipids of the fish rather than in the phospholipids. Fish fed the plant diet compared to fish fed the animal had lower levels of 14:0, 16:1 and 18:1 and higher levels of 16:0 in the

neutral lipids (Table 11. p. 51). The total lipids of the plant diet compared with the animal diet had lower levels of 14:0 (4.18 vs. 7.64%), 16:1 (0.00 versus 9.56%), and 18:1 (4.76 versus 16.13%) and higher levels of 16:0 (30.31 versus 24.08%).

One exception to the fact that differences in saturated and monounsaturated fatty acids between diets were better reflected in the neutral lipids of fish fed the different diets is 18:3ω3. The plant diet had 24.66% 18:3ω3 compared to 0.96% in the animal diet (Table 17). Fish fed the plant diet had more than two times the percentage of 18:3ω3 in their neutral lipids than in their phospholipids (5.12% versus 2.40%, Tables 5 and 11, pp. 41 and 51). The neutral lipids of fish fed the plant diet contained 5.12% 18:3ω3 while that of the fish fed the animal diet contained 1.58% (Table 11, p. 51). The phospholipids of the fish fed the plant diet contained 2.40% of 18:3ω3 compared with 0.41% in fish fed the animal diet (Table 5, p. 41).

In addition, it is hard to explain the higher levels of 20:4ω6 in the phospholipids of fish fed the animal diet (16.54%) compared to fish fed the plant diet (11.13%) based on the respective levels of that fatty acid in the plant versus the animal diet alone (Table 17). However, the animal body is capable of converting any omega-6 fatty acid such as 18:2ω6 to any other omega-6 polyunsaturated fatty acid (Gunstone, 1967; Pearson et al., 1977), and this may be what happened in this case. The total lipids in the alfalfa pellets contained

17.40% of 18:2ω6 compared with 11.18% in the total lipids of the trout chow (Table 17).

The fatty acid composition of the diet did indeed affect the fatty acid composition of the neutral and polar lipids of the fish, but in some instances, it was not an absolute influence in both types of lipids. For simplicity, it is better to look at levels of fatty acid types rather than individual fatty acids in the diets to see this influence. The total percentage of the saturated fatty acids (those containing no double bonds) in the total lipids of the animal and plant diets given in Table 17, are respectively, 40.98 and 51.07. The total percentages of the monounsaturated fatty acids (those containing one double bond) in the animal and plant diets are, respectively, 30.97 and 5.36. The total percentage of the Omega-6 fatty acids (18:2ω6, and 20:4ω6) in the animal diet is 12.13 and in the plant diet, 18.19. The total pecentage of the Omega-6 fatty acids (18:3ω3, 20:5ω3, 22:5ω3, and 22:6ω3) in the animal diet is 15.80 and in the plant diet, 24.66.

Higher levels of the saturated fatty acids in the plant diet compared to the animal diet were reflected in higher levels of these acids in the neutral lipids of the fish fed the plant diet compared to those of fish fed the animal diet (Table 14, p. 56). The opposite of this was observed for the levels of saturated fatty acids in the phospholipids (Table 8, p. 47). The higher monounsaturated fatty acid levels in the animal diet compared with the plant diet resulted in higher percentages of monounsaturated fatty acids in both types of

lipids in the fish fed the animal diet than in those of the fish fed the plant diet (Tables 8 and 14, pgs. 47 and 56). The higher levels of Omega-6 fatty acids found in the plant diet compared with the animal diet also resulted in a higher level of these acids in both types of lipids in fish fed the plant diet compared to those in fish fed the animal diet (Tables 8 and 14, pgs. 47 and 56). As was found with the saturated fatty acids from the diets, the higher levels of the Omega-3 fatty acids from the diets, the higher levels of the Omega-3 fatty acids in the plant diet over the animal diet resulted in higher levels of these acids in the neutral lipids if fish fed the plant diet compared to those fed the animal diet, but not in the phospholipids (Tables 8 and 14, pgs. 47 and 56).

In general, the fatty acids of fish phospholipids were affected by diet as much as the fatty acids of the fish neutral lipids. In the phospholipids, 14 out of 19 fatty acids measured or 72% were significantly affected by feeding time of the two diets (Table 7, p. 43). In the neutral lipids, 17 out of 27 fatty acids measured or 70% were significantly affected by feeding time of the two diets (Table 13, p. 53). These results are in conflict with most researchers of fish lipids. Many researchers (Ackman & Burgher, 1964; Lovern, 1964; Shimma & Taguchi, 1964) have shown that the least variation in the fatty acid composition between species for marine and freshwater fish is in the phospholipids, not the triglycerides. In addition, Halver (1980) reported that the phospholipids are generally considered to be structural or functional lipids, being incorporated to a large extent in the membrane structure of the cell and subcellular particles.

This researcher further indicated that triglycerides are the storage lipids and reflect the fatty acid composition of the diet to a greater extent than do the phospholipids. In another study, Gibson and Worthington (1977) showed that in channel catfish the distribution of fatty acids in phospholipids was influenced by dietary lipids (menhaden and tallow oil) but not to the extent observed in triglycerides.

Fatty Acid Composition of Fish Phospholipids Versus Neutral Lipids

The phospholipids of grass carp fed either diet were richer in polyunsaturated fatty acids than the neutral lipids (Table 5, p. 41, and Table 11, p. 51). Nair and Gopakumar (1984) found similar results with five species of lean fish and three species of shell fish. The only exception was in fresh water prawns; their neutral lipids had higher levels of polyunsaturated fatty acids than the phospholipids.

Both the neutral lipid and phospholipids of grass carp fed either diet had relatively high levels of 16:0 (Tables 5 and 11, pp. 41 and 51). Ackman (1967) found that the oil of fresh water and marine fish was high in the percentages of palmitic acid.

Sensory Evaluation of Grass Carp

The results of the consumer panel for palatability characteristics of catfish and white amur fed different diets are presented in Table 18. There were no significant differences ($P<0.05$) in sensory scores between white amur fed alfalfa pellets (Treatment 1) and catfish (Treatment 3) or between white amur fed the 2 different diets (Treatments 1 and 2). No significant differences were found among

Table 18. Mean Sensory Scores[a] for Palatability Characteristics of Catfish and White Amur Fed Different Diets.

Characteristic	Treatment[b] 1	2	3
Flavor	3.57[cd]	3.37[c]	3.72[d]
Texture	3.37	3.39	3.59
Acceptability	3.43[cd]	3.28[c]	3.63[d]

[a]n = 93; 5 point scale where 1 = moderately negative feeling and 5 = very positive feeling.

[b]1 = white amur fed alfalfa pellets; 2 = white amur fed trout chow pellets; 3 = commercially produced catfish.

[c,d]Means in a row bearing inlike superscripts are different at the P<.06 level.

treatments for texture. White amur fed trout chow pellets had a less desirable flavor and lower acceptability than did catfish. Bakir (1985) found similar results when he compared the palatability characteristics of white amur fed three different diets (trout chow pellets, bermuda grass pellets and sudan grass) and catfish, in that the fish fed the plant diets were more acceptable than those fed trout chow and the fish fed plant diets were as acceptable as the catfish. The present study and the study by Bakir (1985) are contrary to what Wilson and Cottrell (1979) found, in which grass carp were equally preferred or preferred over channel catfish.

Since no significant difference was found in the flavor score between grass caarp fed the animal diet and those fed the plant diet, the differences found in the fatty acid compositions of the fish fed these two diets did not cause any great flavor problems. The fatty acid type most likely to cause flavor problems in fish would be the Omega-3, particularly 18:3ω3 fatty acid, which has been reported to produce off-flavors such as grassy, fishy and painty in soybean oil upon oxidation (deMan, 1985). However, the plant diet which contained a higher level of Omega-3 type fatty acids than the animal diet (Table 17, p. 60) produced fish that tended to have a higher flavor score than the fish produced by the animal diet. The facts that the fish fed the animal diet had a higher level of total lipids after 6 months feeding time than fish fed the plant diet (2.23 vs 0.73%) and that the phospholipids of the fish fed the animal diet contained higher levels of omega-3 polyunsaturated fatty acids than those of

fish fed the alfalfa pellets (30.39 vs 24.51%) must be taken into consideration when discussing the total levels of omega-3 fatty acids in the fish. Also, the levels of omega-3 fatty acids in neutral lipids of the plant fed fish were not that much higher than those of the animal fed fish (15.42 vs 12.91%). It is possible because of these facts that the fish fed the animal diet may have had similar or even higher levels of omega-3 fatty acids than the fish fed the plant diet which tended to cause the animal-fed fish to have a lower flavor score than the plant fed fish.

The most serious problem in the present study and the study by Bakir (1985) was the presence of intramuscular bones in the white amur fillet. The fish in the present study had an average weight of 1448 g compared with an average weight of 750 g in the study of Bakir (1985), but the bones were still present in enough samples to be cited as the major reason by the panelists for scoring the grass carp lower than they would have, had the bones been absent. These results indicate that the grass carp need to be bigger than 1448 g at market size, and any bones must be removed from the fillets prior to marketing. Perhaps some type of mechanical system can be developed to produce boneless fillets from the grass carp. If the problem of the bones could be solved either by producing larger fish or by some type of mechanical processing, then the grass carp would indeed be choice food fish.

Results of Questionnaire from the Fish Consumer Panel

The results (76%) of the 93 consumer panelists who answered the questionnaire were male. The ages of the panelists ranged between 18 and 45. Only one panelist was over 45 years old. The majority of the panelists (81%) were in the age range between 18-24 years, 14% of the panelists were 25-34 years old, and 4% of the panelists were in the age range between 35-45 years.

Panelists lived mainly in 3 types of residences. Thirty-five percent of the panel lived in a dormitory, sorority, or fraternity, 23% lived in single family dwellings, and 24% lived in multiple-family dwellings (duplex, apartment, townhouse). The lowest percentage of the respondents (13%) lived in a rented room. The two main sources of the panelists' meals were at home (57%) and at a cafeteria (33%). Most of the consumer panelists (70%) had never lived near the coastline or near a major body of water.

Many of the respondents (57%) ate fish one or more times per month, and 33% of the respondents ate fish one or more times per week as shown in Table 19. Sanchez and Konopa (1974) found that there were two kinds of users of fresh finfish: regular and irregular users. They defined the regular user of fresh finfish as consumers who used fresh finfish at home one or more in a month and irregular users as consumers who used fresh finfish less than once a month. Therefore, in this study, the majority of the consumers (90%) were regular users since they ate fish more than once a month. However, liking fish was not a criterion for participating on this panel for evaluating fish.

Table 19. Frequency of Eating Fish by Consumer Panel.

Eating Frequency	% of Panel[a]
One or more times per week	33
2-3 times per month	57
Once per month	2
6-10 times per year	4
Less than 6 times per year	1
Never	2

[a] n = 93.

Madeira and Penfield (1985) reported the attitudes and demographic characteristics of 39 panelists who evaluated fish in their study, but the panelists in their study had to like fish in order to serve on the panel. In the present study, with a high percent of regular fish users on the panel evaluating the grass carp and answering the questionnaire, an understanding concerning the consumption of both utilized/underutilized, traditional/nontraditional fish, and the acceptance by the consumer of both fresh and frozen underutilized fish species may be obtained. Lewis and Shoemaker (1986) emphasized that consumer attitude concerning familiarity with seafood played an important role in allowing the consumer to try nontraditional fish, and that people who do not like fish in general will never try nontraditional fish. Therefore, in this present study, the majority of the panelists, because of their familiarity with such salt water species as cod, flounder, haddock, halibut, and tuna (Table 20), should evaluate fish such as grass carp without bias.

It was interesting to find in this study that more than half (51%) of the consumer panel ate fish prepared at home more than at fast food restaurants or cafeterias (23%), or full service restaurants (17%) as shown in Table 21. These results are contrary to Lewis and Shoemaker (1986) who found that most people like to go to restaurants to eat fish and also contrary to Madeira and Penfield (1985), who found that most of their respondents preferred to eat fish at the home of a friend or relative. As shown in Table 21, 3% of the panelists specified the "other" category where they bought fish from the

Table 20. Type of Fish Usually Prepared at Home by the Consumer Panel[a].

Fish Type	% of Panel Who Prepared the Fish at Home
Catfish	49
Flounder	45
Salmon	34
Perch	33
Cod	19
Haddock	11
Other[b]	40

[a] n = 93.

[b] Assign to bass, bream, bluegill, crappie, grouper, north pike, snapper, sword fish, trout, tuna, walleye.

Table 21. Places Where Most Frequently Prepared Fish Was Consumed by the Consumer Panel[a].

Places Where Fish Prepared	% of Panel
At home	51
Fast-food restaurant or cafeteria	23
Full-service restaurant	17
Home of a friend or relative	7
Other[b]	3

[a] n = 93.

[b] Refers to supermarket, frozen fish, when camping.

supermarket or bought frozen fish which, in fact, could be included as home preparation, increasing the percentage of the panelists to 54% for preferring preparation of fish at home.

Answering questions 9 through 12 of the questionnaire (Appendix B) was dependent on a positive answer to preparation of fish in the home (question 8). Eighty-nine percent (83 of 93) of the panelists answered question 8 positively. The majority of these respondents (68 of 83) preferred fish in the form of fillets (Table 22). The second most preferred form was whole fish (24 of 93 respondents), and the third choice was as preformed fishsticks, croquettes, etc. This information indicated that when a new species of food fish is introduced to the market, the best form to sell is fillets.

The methods in which the consumers prepared fish in their homes were varied. Sixty-two of the 83 panelists fried fish at home, 41 broiled fish at home, and 33 baked fish (Table 23).

The percentage of the consumer panelists who prepared particular species of fish at home are shown in Table 20. Catfish, flounder, salmon, and perch were cited as the most used species.

There are many reasons for not preparing fish at home, and the reasons cited by the consumer panel in this study are given in Table 24. The reasons: do not think of it, kind and/or form I like are not readily available, and cost were the most cited for not preparing fish at home. This agrees partially with the findings of Madiera and Penfield (1985) except in their study consumers put the lack of availability of the kind and/or form I like as the main reason for not

Table 22. The Ways the Panelists Prefer to Prepare a Fish in Their Homes as Reported by Part of the Consumer Panel.

Type of Fish Preparation	% of Total Number of Panelists Who Answered Question
As fillets	68
As whole fish	24
Preformed (fishsticks, croquettes, etc.)	10
Other[b]	1

[a]10 of 93 panelists did not prepare fish in their homes, and therefore did not answer the question.

[b]Assign to canned tuna.

Table 23. The Type of Cooking Method Usually Used by Panelist to Prepare Fish in Their Homes as Reported by 83 Panelists[a].

Type of Cooking Method	Number of Panelists
Fried	62
Broiled	41
Baked	33
Steamed	5
Other[c]	10

[a]10 of 93 panelists did not prepare fish in their homes.

[b]Refers to canned tuna, and grilled or deep-fried fish patties.

Table 24. Important Reasons for Not Preparing Fish More Frequently at Home as Reported by Part[a] of Consumer Panel.

Reason	% of Total Number of Panelists Who Answered the Question		
	First Most Important Choice	Second Most Important Choice	Third Most Important Choice
Don't think of it	24	6	12
Kind and/or form I like is not readily available	18	16	5
Cost too much	11	8	6
Other[b]	8	7	5
Don't like its taste and/or texture and/or odor	6	3	3
I am unfamiliar with ways to prepare fish	4	11	3
Unfamiliar with fish, generally	3	2	7

[a]83 out of 93 panelists answered question.

[b]The reasons cited were husband does not like fish, difficult to prepare fish, do not like to clean them, prefer beef, etc.

preparing fish, and in the present study, the main reason was that they do not think of it. In both studies, cost was considered in the consumer not preparing fish more often. Capps (1982) reported that a 10% increase in the price of fish and shellfish resulted in a 5.32% decrease in fish and shellfish expenditure.

Respondents were asked to indicate their familiarity and experience with specific fish, including the fish used in this study, white amur or grass carp. With the exception of white amur, orange roughy, buffalo, and monkfish, more than half of the panel participants indicated they had heard of each of the other different types of fish (Table 25). White amur was the least known fish of those listed in the survey. The fish that most of the panel (29) had heard about was flounder. The fishes that were recognized by the next largest number of respondents (90) were salmon, tuna, and catfish. The fishes that most of the panel liked to eat were tuna (75 respondents), catfish (73 respondents), and salmon (71 respondents). The least liked fish for eating was buffalo (Table 25).

The name of the fish plays an important role in its marketability according to Martin et al. (1983). However, Lewis and Shoemaker (1986) found in their study that the name of the fish only inhibited 34% of the consumers from trying non-traditional fish. In the present study, the two names for the same fish, white amur and grass carp, also affected the consumer's attitude toward and recognition of the fish. In the case of grass carp, 51 of the panelists had heard of it and only 21 had heard of white amur. Four panelists

Table 25. Frequency and Percentage of Consumer Panels Who Reported the Familiarity With, Eaters of, Where Prepared, and Like/Dislike of 15 Species (Types) of Fish[a].

Fish Type[a] (Common Name)	Number of Respondents (n = 93)		% of Consumer Panel Who Have Eaten			
	Heard of	Eaten	Prepared at Home	Prepared at Restaurant	Like	Dislike
Monkfish	35	13	46	54	62[b]	15[b]
Catfish	90	79	77	66	90[b]	6[b]
Cod	88	57	50	60	72[b]	16[b]
Carp	89	26	50	23	50	50
Flounder	92	79	62	75	92	3
White Amur	21	6	83	17	83	17
Haddock	66	25	60	60	92[b]	8
Redfish	59	29	31	66	76[b]	7[b]
Halibut	80	33	39	79	82[b]	12[b]
Orange Roughy	22	12	58	50	83[b]	0[b]
Salmon	90	75	84	33	80	15[b]
Grass Carp	51	4	100	25	50[b]	50
Buffalo	35	5	60	0	20[b]	60[b]
Brim	75	56	88	14	89[b]	9[b]
Tuna	90	81	88	32	93	10[b]

[a]There are 14 types of fish since white amur and grass carp are the same fish.

[b]Some did not respond when appropriate.

said that they had eaten grass carp and only 2 reported they liked it. Six of the respondents reported they had eaten white amur and 4 said they liked it. The name, white amur, seems to draw a positive reponse from many people compared to a negative reaction to the name, grass carp. This negative reaction results in a lower value of the fish to consumers who have never even tasted the fish. This attitude most likely is the result of common carp being rated as a trash fish in the U.S.A. Eighty-nine panelists in this study had heard about carp; only 26 had eaten common carp, and only 50% of those reported they liked it. If the grass carp is to become a food fish, it most likely will have to be marketed as white amur.

CHAPTER V

SUMMARY

The effect of two fish diets, trout chow pellets and alfalfa pellets, were determined on the growth, chemical components and sensory characteristics of white amur or grass carp during a 6 month feeding trial. The following chemical components: level and composition of lipids and fatty acid composition of neutral and polar lipids, were determined on fillets of the fish at 0, 2, 4, and 6 months feeding time. At the end of the 6 month feeding period, a consumer panel of 93 people evaluated the flavor, texture, and acceptability of fillets of the white amur fed the alfalfa pellets (plant diet) and the trout chow pellets (animal diet) in comparison with commercially produced catfish fillets. In addition, the total lipid level and fatty acid composition of each diet were determined.

Prior to the start of the feeding trial, white amur weighing an average of 938 g per fish were acclimated two weeks to living in laboratory tanks and conditions under which the experiment was conducted. At the beginning of the experiment, 3 fish selected at random were killed and fillets removed as the 0 month samples, and twelve fish were assigned to each of 6 tanks. Fish in 3 tanks were fed the plant diet, and fish in the other 3 tanks were fed the animal diet. The diets were fed at 2% of total body weight of the fish, two times per day for 6 days a week throughout the experiment. Water temperature

in the tanks was maintained at 24 ± 2° C, and oxygen, ammonia and chlorine levels in the water were maintained at acceptable levels. At 2, 4, and 6 months feeding time, two fish were removed from each tank, killed and their fillets removed for chemical analyses.

Fish fed trout chow pellets gained weight faster during the 6 months than did fish fed alfalfa pellets. Fish fed trout chow pellets for 6 months weighed an average of 1585 g compared with 1325 g for the fish fed the alfalfa pellets. Fillets of fish fed the trout chow also increased in fat content from 1.80% at 0 month to a maximum of 3.72% at 4 months feeding time while that in fillets of fish fed the alfalfa pellets tended to decrease. The phospholipids of the fish fillets were composed of 6 to 13% sphingomyelin, 55 to 63% lecithin and 26 to 35% cephalin. Levels of the phospholipid classes were significantly affected by feeding time. The percentage of sphingomyelin decreased linearly in fish fed the animal diet with increasing feeding time and had a cubic relationship with feeding time in fish fed the plant diet. The level of lecithin decreased from 62.7% in the fillets of fish at 0 months to a minimum level of 54.8% at 4 months feeding time before increasing to 62.0% at 6 months ($P < 0.05$). In contrast to this, the level of cephalin increased from 26.7% at 0 months to a maximum of 34.9 at 4 months feeding time and decreased to 30.8% at 6 months ($P < 0.05$).

Nineteen fatty acids were identified in the phospholipids of the fish: 14:0, 16:0I, 16:1, 18:0, 18:1, 18:1I, 18:2ω6, 18:3ω6, 18:3ω3, 20:1, 20:2, 20:3, 20:4ω6, 20:5ω6, 22:5ω6 and 24:1. Significant

differences between diets were found in the percentages of 14 of these fatty acids, and feeding time also significantly affected the percentage of 14 of these fatty acids. In general, fish fed the trout chow had higher percentages of saturated, monounsaturated and omega-3 polyunsaturated fatty acids and lower percentages of omega-6 polyunsaturated fatty acids in the phospholipids than did fish fed the alfalfa pellets. The percentage saturated fatty acids in the phospholipids of fish fed both diets decreased with increasing feeding time from 0 to 4 months; however, these acids increased in percentage more from 4 to 6 months in fish fed the trout chow than in fish fed the alfalfa pellets. The percentage monounsaturated fatty acids in the phospholipids of fish fed both diets decreased linearly from 15.50 at 0 month to 13.35 at 6 months feeding time when averaged across diets ($P < 0.05$). With increasing feeding time from 0 to 6 months, the percentage of omega-6 polyunsaturated fatty acids in the phospholipids decreased linearly from 26.01 to 15.54 in fish fed the trout chow but increased linearly from 26.01 to 32.77 in fish fed the alfalfa pellets ($P < 0.05$). In fish fed the trout chow the percentage omega-3 polyunsaturated fatty acids in the phospholipids increased linearly from 21.52 to 34.73 with increasing feeding time from 0 to 6 months while the percentage of these acids in fish fed alfalfa pellets increased quadratically from 21.52 to 32.51 ($P < 0.05$).

Twenty-seven fatty acids were found in the neutral lipids of the grass carp fillet. All of the fatty acids found previously in the phospholipids except 16:0I were also found in the neutral lipids

plus 9 additional fatty acids: 16:1I, 17:0, 17:1, 20:0, 22:1, 23:0, 24:0, 24:4 and an unknown. Significant differences between diets were found for 18 of these acids, and feeding time significantly affected 19 of these acids. Fish fed trout chow had lower percentages of saturated, omega-6 polyunsaturated and omega-3 polyunsaturated fatty acids and a higher percentage of monounsaturated fatty acids in the neutral lipids than did fish fed alfalfa pellets ($P < 0.05$). The percentage of saturated fatty acids in the neutral lipids increased linearly from 31.36 to 38.05 from 0 to 6 months in fish fed alfalfa pellets, but was not affected by feeding time in fish fed trout chow ($P < 0.05$). The percentage of monounsaturated fatty acids in the neutral lipids was not significantly affected by feeding time in fish fed either diet, but the percentage of omega-3 polyunsaturated increased linearly with increasing feeding time in the neutral lipids of fish fed both diets. In fish fed the trout chow, the percentage of omega-6 fatty acids in the neutral lipids of fish fed the trout chow decreased quadratically from 27.64 at 0 months feeding time to a minimum of approximately 14.28 at 4 months before increasing slightly from 4 to 6 months. In the fish fed alfalfa pellets the percentage of these same acids increased from 27.64 at 0 months to 30.24 at 2 months and then decreased to a minimum of 23.97 at 4 months before increasing to 26.13 at 6 months feeding time ($P < 0.05$).

In comparing the effect of the plant versus the animal diet on the fatty acids composition of the phospholipids and neutral lipids of the grass carp fed the two diets, the following results were found.

Higher levels of saturated fatty acids in the plant diet were reflected in the higher levels of these acids in the neutral lipids but not the phospholipids of the fish fed the plant diet compared with those fed the animal diet. The higher percentages of the monounsaturated acids in the animal diet resulted in higher percentages of these acids in both types of lipids in fish fed the animal diet compared with those of fish fed the plant diet. Higher percentages of the omega-3 polyunsaturated fatty acids in the plant diet resulted in higher levels of these acids in both types of lipids in fish fed that diet compared with those of fish fed the animal diet. Although the plant diet contained higher levels of omega-3 polyunsaturated fatty acids than the animal diet (24.66 vs 15.80%), only the neutral lipids of fish fed the plant diet had higher levels of these acids than those of fish fed the animal diet. The reverse of this was true for the phospholipids.

Ninety-three consumers, the majority of whom were between 18 and 24 years of age (81%), evaluated fried, battered fillets of white amur fed the animal diet, white amur fed the plant diet and channel catfish without knowing the identity of the fish samples they tasted. Ninety percent of these consumers could be classified as regular users of fresh fish in that they ate fish more than once a month. Fifty-one of the panelists reported that they had heard of grass carp and only twenty-one had heard of white amur. Only four consumers reported they had eaten grass carp and two said they did not like it. Six consumers reported that they had eaten white amur and four said

they liked it. The consumers scored the flavor of the grass carp fed the plant diet almost as high as that of the catfish (3.6 vs. 3.7, respectively, on a 5 point scale where 3 = slightly positive feeling and 4 = moderately positive feeling), but they scored the flavor of grass carp fed the animal diet (3.4) significantly lower than that of catfish. They could not tell the difference in the texture among the three fish samples, but the small bones in the fillets were the reason given for scoring the acceptability of the grass carp samples lower than that of the catfish.

Results of this study indicate that fish diet affects the fatty acids of phospholipids as much as or more than that of the neutral lipids; this is in disagreement with results reported in the literature. The fatty acid composition of the diet also played an important role in determining the types and levels of fatty acids found in fish lipids. Diet was also found to affect the flavor of the grass carp; however, the plant diet compared to the animal diet had the highest level of omega-3 polyunsaturated fatty acids and produced the grass carp that had the best flavor. This might not be expected since these fatty acids have been associated with off-flavors in other foods and in causing the fast development of rancid flavors in other fish products. The flavor of the grass carp fed the alfalfa pellets was scored as high as that of catfish which indicates that grass carp has a future as a food fish. However, a problem of small bones in the fillets of grass carp weighing approximately 1325 to 1585 grams indicates that the carp must be grown to a larger size or

processed in a way to remove these bones for the fish to really be successful in the market. The fish would also probably be better received by consumers if they were marketed under the name white amur rather than grass carp.

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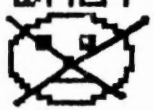
APPENDIXES

APPENDIX A

FISH SCORECARD

SAMPLE NUMBER _____

FISH SCORECARD

Please tell us what you think about this fish Mark the face  that best represents how well you like each characteristic as well as how you like it overall.

Flavor					
Texture					
Overall					

APPENDIX B

QUESTIONNAIRE

1. Gender: _____Male _____Female

2. Age:

_____under 18 years

_____18-24 years

_____25-34 years

_____35-45 years

_____Over 45

3. Type of residence:

_____single family dwelling

_____multiple-family dwelling (duplex, apartment, townhouse)

_____mobile home

_____dormitory, sorority, fraternity

_____rented room

_____other, please specify _____

4. What is the main source of your meals? (Please check only one)

_____cafeteria

_____home (prepared by yourself and/or someone else)

_____home of friends or relatives

_____fast-food restaurants or shops

_____full-service restaurants

_____other, please specify _____

5. Did you ever live near the coastline of a major body of water (Great Lakes, oceans) where a variety of fish was readily available?

_____yes Please specify area_____

_____no

6. Check the time period which best describes the frequency with which you eat fish. (Please check only one)

_____one or more times per week

_____2-3 times per month

_____once per month

_____6-10 times per year

_____less than 6 times per year

_____never

7. If you eat fish, where is it most frequently prepared? (Please check only one)

_____at home

_____home of a friend or relative

_____fast-food restaurant or cafeteria

_____full-service restaurant

_____other, please specify _____

8. Do you (or someone else) prepare fish (not shellfish) in your home?

_____yes _____no

IF THE ANSWER TO QUESTION 8 IS "YES", PLEASE ANSWER ALL OF THE FOLLOWING QUESTIONS
IF NOT, GO DIRECTLY TO QUESTION 13 AND CONTINUE.

9. In what form is fresh and/or frozen fish (not shellfish) most frequently prepared in your home (check all that apply)

_____as whole fish

_____as fillets

_____preformed (fishsticks, croquettes, etc.)

_____other, please specify _____

10. By what cooking method(s) is fish prepared in your home? (please check all that apply)

_____baked

_____broiled

_____fried

_____steamed

_____other, please specify _____

11. What kind(s) of fresh and/or frozen fish are usually prepared in your home? (check all that apply)

_____cod

_____haddock

_____perch

_____flounder

_____catfish

_____salmon

_____other, please specify _____

12. From the list below, please choose the three most important reasons why fish is not prepared more frequently in your home. Place a 1 next to the most important, 2 next to the second most important reason, and 3 next to the third most important reason.

_____It costs too much

_____The kind and/or form I like is not readily available

_____I am unfamiliar with fish, generally

_____I am unfamiliar with ways to prepare fish

_____I don't like its taste and/or texture, and/or odor

_____I don't think of it.

_____other, please specify _____

13. Please check each column that applies for each fish (fresh and/or frozen) listed below.

	(a) have heard of	(b) have not heard of	(c) have eaten	(d) was prepared at home	(e) was prepared in restaurant	(f) I liked it	(g) I did not like it
monk fish	____	____	____	____	____	____	____
catfish	____	____	____	____	____	____	____
cod	____	____	____	____	____	____	____
carp	____	____	____	____	____	____	____
flounder	____	____	____	____	____	____	____
White Amure	____	____	____	____	____	____	____
haddock	____	____	____	____	____	____	____
red fish	____	____	____	____	____	____	____
halibut	____	____	____	____	____	____	____
orange roughy	____	____	____	____	____	____	____
salmon	____	____	____	____	____	____	____
grass carp	____	____	____	____	____	____	____
buffalo	____	____	____	____	____	____	____
brim	____	____	____	____	____	____	____
tuna	____	____	____	____	____	____	____

VITA

Hussain M. Bakir Al-Hakkak, son of Mohammad Bakir Al-Hakkak, was born on July 1, 1948, in Baghdad, Iraq. He graduated from Al-athamia High School, Baghdad, Iraq, in 1969. He began his university study in 1970 at University of Baghdad in the College of Science, Department of Zoology and Microbiology. He graduated in 1973 and received a Bachelor of Science degree in Biology. He served for one year beginning in June 1974 with the Ministry of Health, Medical City in Baghdad, as a research assistant. In June 1975 he joined the University of Baghdad, College of Veterinary Medicine, as an instructor in the Bacteriology Laboratory.

In June 1979 he received a scholarship from the Iraqi Government to study in the United States. In January 1980 he enrolled in the Department of Biology at Northeast Missouri State University to study for the Master of Science degree. He received the M.S. in Biology in May 1982. In June 1983 he enrolled in the Department of Forestry, Wildlife, and Fisheries at The University of Tennessee, Knoxville. He received the M.S. degree in Wildlife and Fisheries Science with major emphasis in fisheries (aquaculture) in August 1985. In September, 1985, he enrolled in the Department of Food Technology and Science at The University of Tennessee, Knoxville. He received the Doctor of Philosophy degree in Food Technology and Science with major emphasis in Food Chemistry in August, 1987.

He married Sajida S. Jafar, daughter of Sadik Jafar Al-Bajey, Baghdad, Iraq on July 1, 1976. They have three lovely children: Mariam, born June 10, 1977, Mostafa, born December 18, 1978, and Belal, born August 11, 1982.