AN ABSTRACT OF THE THESIS OF

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(Name) (Degree) (Major)

Date thesis is presented March 17, 1967

Title SOME EFFECTS OF CYCLOPROPENOID FATTY ACIDS ON LIPID METABOLISM IN RAINBOW TROUT (SALMO GAIRDNERII)

Abstract approved _____
(Major Professor)

Diets containing cyclopropenoid fatty acids (CPFA) were fed to rainbow trout. At a level of 223 ppm (from Sterculia foetida oil), these CPFA in six weeks reduced weight gain by as much as 50 percent over the control fish on the same diet without CPFA. Compounds containing the intact cyclopropene ring were recovered from the tissue lipids of the trout at approximately two-thirds of the level fed. These acids seemed to concentrate in the egg lipids of the adult female fish. At 223 and 2233 ppm (from S. foetida oil) and 50 ppm (from food grade cottonseed flour) the CPFA were demonstrated to alter lipid metabolism. In general, the CPFA fed fish had higher stearic and palmitic acid levels and lower oleic and palmitoleic acid levels in their tissue lipids than did the controls. On diets containing corn oil or corn oil

plus salmon oil, fish fed CPFA tended to deposit more long chain unsaturated fatty acids than did their controls. When provided with tristearin as the sole dietary lipid, the CPFA fed fish reduced the level of unsaturation of their tissue lipids.

SOME EFFECTS OF CYCLOPROPENOID FATTY ACIDS ON LIPID METABOLISM IN RAINBOW TROUT (SALMO GAIRDNERII)

by

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1967

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Date thesis is presented March 17, 1967

Typed by Bernice Caceres

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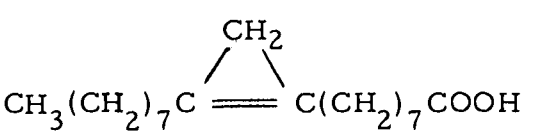
INTRODUCTION

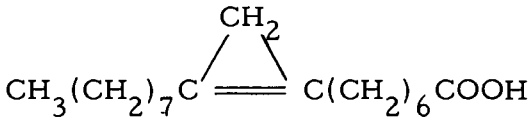
The widespread use of cottonseed oil for food and cottonseed meals for feeds, coupled with recent reports of serious metabolic alteration brought about by constituents of the cottonseed, stimulated the research of this thesis. The study was undertaken to investigate some of the effects of cyclopropenoid fatty acids, naturally occurring chemical oddities present in cottonseed oil, on the metabolism of rainbow trout.

Two cyclopropenoid fatty acids (CPFA[1/]), sterculic and malvalic acids, have been found to occur in the triglyceride fraction of nearly 50 different plant oils (39). In 1952, the structure of sterculic acid, isolated from Sterculia foetida (java olive) oil where it constitutes approximately 50 percent of the lipid portion, was established by Nunn (37) to be:



1/ Although this designation is used here for the free fatty acids, it is retained throughout the remainder of the text to represent the fatty acids containing a cyclopropene function, whether free or in triglyceride form.

It was not until 1964 that Craven and Jeffries (9) determined the structure of dihydromalvalic acid, and based on Nunn's sterculic acid structure, assigned malvalic acid the following structure:



The presence of shorter chain CPFA has been implicated by Shenstone and Vickery (54), but none have been isolated or identified to date.

The double bond in the strained three-membered ring in these compounds gives them a great potential of chemical reactivity: This site can be readily attacked by a number of nucleophilic reagents including the carboxyl group of another CPFA molecule to give a polymerization product (7). This site can also react readily with many sulfhydryl containing reagents (26); this reaction is the basis of several chemical determinations for the CPFA (12, 20, 40). The reaction of CPFA with the sulfhydryl groups of proteins in animal systems has been thought to be important in their biological action (26, 38). In spite of their extreme reactivity, these compounds can be quite stable in living systems and may even withstand the severe processing conditions which occur during the extraction and refining of the oil. At least part of the explanation for the unusual stability of such labile compounds may be attributed to the protectivity of

their natural triglyceride environment which would hinder reactions of the type to destroy the cyclopropene ring, which is necessary for assay by all available procedures. The natural stability of these compounds is demonstrated in this research: CPFA can be removed from the plant source, compounded into diets and detected in the tissues of the test animals at levels more than two-thirds of that fed in the animal diet.

This thesis was designed to show metabolic changes brought about by the short term feeding of CPFA to rainbow trout. Changes in growth, lipid deposition and fatty acid composition, including tissue build-up of CPFA were followed. Saturated lipid diets were employed to provide additional stress in one feeding trial. Extended feeding studies were carried out to demonstrate long term effects of the CPFA on the fatty acid composition of various organs of the trout.

REVIEW OF LITERATURE

Biological Effects

Poultry

Early research in poultry science by Thompson (59) to determine why the whites of cold storage eggs sometimes showed a pink discoloration developed into an intensive investigation of dietary components. Sherwood (55) reported that the defect was caused by inclusion of cottonseed products in the diets of layers. Lorenz et al. (29, 30) hypothesized that it was due to the Halphen reactive component(s) of cottonseed oil. Shenstone and Vickery (52) and Masson et al. (33) later produced identical disorders by feeding isolated sterculic and malvalic acids. Nordby (36) found that it was necessary to feed compounds containing the intact cyclopropene ring to produce pink discoloration. He also revealed that all yolks from the discolored eggs were Halphen positive while none of the normal yolks were. Schaible and Bandemer (46) determined that the discoloration was due to a conalbumin-iron complex formed by iron which migrated across the vitelline membrane to the white where it chelated with conalbumin. Doberenz et al. (11) demonstrated that the discolored eggs had very viscous whites and pasty yolks of higher water content and correspondingly larger size than normal. Various

workers (7, 39, 52) have attributed the above changes to alteration of the permeability of the vitelline membrane by the highly surface active CPFA. This theory has also been applied to explain other changes noted in eggs, including increased nonprotein nitrogen content (primarily amino acids) of the whites (52, 53), increased iron in the whites (52, 53) and selective protein transfer across the membrane with ovalbumin, conalbumin and lysozyme being transferred to the yolks while livetin migrates to the white (13, 14).

The hypothesis of membrane permeability alteration may not be sufficient to explain the observed egg abnormalities. Shenstone and Vickery (53) modified the theory when they observed CPFA combined in the yolk lipids rather than in the membrane; they hypothesized the mode of action of CPFA was through alteration of the yolk emulsion structure or boundary layer instead of in the membrane per se.

Alteration of the reproductive process in poultry by CPFA has been observed by various workers. Dietary CPFA decreased egg production and eventually caused cessation of laying (53). Depression of egg hatchability was also noted and thought to be due, at least in part, to increased chick embryo mortality (47). In addition, feeding CPFA to immature chickens delayed sexual maturity and caused enlargement of various organs (48, 51).

Another aspect of this problem, perhaps quite closely related

to the effects already mentioned, is the interference of CPFA with lipid metabolism. Hens on CPFA diets deposited cyclopropene ring-containing compounds in their egg lipids (31, 36) and depot fat (1, 30) as evidenced by positive Halphen tests of the samples. Malvalic or sterculic acids *per se* have not yet been identified in animals ingesting the CPFA. The dietary CPFA are able to bring about a dramatic alteration of the fatty acid composition of both the egg and the tissue lipids of hens, with a significant shift towards saturation. Evans *et al.* (15) found that feeding CPFA increased the stearic acid and reduced the oleic acid deposited, most noticeably in the yolk lipids, significantly in the liver and plasma lipids and slightly in the depot fat and heart lipids. Reiser *et al.* (42) attributed such a shift to an inhibition of the fatty acid dehydrogenating enzyme(s) which are believed to possess thiol groups at active sites. Kircher (26) carried out *in vitro* experiments which proved that CPFA could react with thiol compounds. Ory and Altschul (38) demonstrated that CPFA could inhibit the action of sulfhydryl-containing castor lipase as much as 50 percent. Reiser's group then hypothesized that CPFA may be capable of reacting *in vivo* with enzymes possessing active sulfhydryl groups (42) and with subsequent experiments (43) supported their hypothesis by inhibiting the stearate to oleate conversion in rats using CPFA.

Mammals

Phelps et al. (39) reviewed the biological effects of CPFA in various mammals. Alteration of the fatty acid composition was noted in the back fat of hogs and the butterfat of cows consuming cottonseed products. The relative percent of stearic acid was elevated while that of oleic acid was depressed. Experiments on the effects of CPFA on reproduction have been carried out with rats and mice. Braden and Shenstone (6) showed that mice failed to exhibit observable changes in fertility or mean litter size even when fed diets containing ten times the amount of CPFA deleterious to hens. Sheehan and coworkers (49, 50), however, demonstrated that CPFA inhibited sexual maturation with a corresponding decrease in fertility in female rats. More recent work has revealed histomorphological changes in the reproductive tissues of the female rat, with the male being relatively unaffected (41).

Fish

Limited studies suggest that CPFA induce fatty acid shifts in fish similar to those reported for other animals. Reiser and coworkers (42) found an increase in the stearic acid content in one Fundulus but also noted an increase in oleic acid. Decreases in long chain unsaturates compensated for these increases. Sinnhuber'

(56) in preliminary investigations found that various histomorphological changes occur in liver cells of rainbow trout fed CPFA. A recent report by this group on the cocarcinogenicity of CPFA with aflatoxin B_1 in rainbow trout described the detrimental relationship of these two substances associated with the feeding of cottonseed products (57).

Fish as Test Animals

Although relatively little work has been published on lipid metabolism in fish, considerable research has been carried out with other laboratory animals in this area. Limited metabolic studies with various species of fish provide considerable evidence that fatty acid metabolism is quite similar (perhaps identical) to that in the mammals and birds; They deposit dietary lipids, require certain essential fatty acids, and convert fatty acids and other non-lipid compounds to meet body requirements via beta-oxidation and other metabolic pathways. However, lipid metabolism in fish does exhibit one marked difference from that in warm-blooded laboratory animals. Most fish deposit in their triglycerides and more notably in the phospholipids, many long chain, highly unsaturated fatty acids. These are characteristic of fish lipid and essential to the well being of the animal (19). The long chain unsaturates contribute significantly to the overall high level of unsaturation in fish lipids.

Previous work on the biological action of CPFA, reviewed by Phelps et al. (39), has emphasized their interference with lipid metabolism, especially increasing the deposition of saturated fatty acids. Fish, because of the natural occurrence of many long chain unsaturates, could be expected to show this fatty acid shift in greater magnitude. They would be expected also to suffer more from any such change; since being poikilotherms, low environmental temperature would cause them to be extremely sensitive to increasing saturation of tissue lipids.

Other characteristics of fish that enhance their usefulness as test animals for this study are that many offspring can be obtained from a single mating, thus minimizing biological variation among the experimental animals, and large numbers of animals can be maintained in essentially the same environment with minimal care and cost.

From an economic standpoint, these studies of the action of CPFA have a practical value in that CPFA containing products (cottonseed) are good protein sources and may be used in the rations of hatchery-reared fish.

Analysis Techniques

Fatty Acid Identification and Measurement

In lipid research, gas-liquid chromatography (GLC) is one of

the most widely used techniques for the separation, identification and measurement of the methyl esters of fatty acid mixtures. A large number of known standards and standard mixtures of esters are commercially available for comparison with experimental samples. For the more unusual and less available esters, a number of retention times and equivalent chain lengths on various columns have been published. Other methods are available for the quantification and qualitation of fatty acids in mixtures, but were not used in this research.

Cyclopropenoid Fatty Acid Measurement

There are several different procedures available for the quantitative determination of CPFA; all of these procedures are based on the measurement of the amount of intact cyclopropene ring in the sample. In 1897, Halphen (20) reported that cottonseed oil reacted with a mixture of sulfur in carbon disulfide and isoamyl alcohol producing a red color. Although the exact nature of the pigment has not been fully elucidated, Zahorszky and Rinehart (63) have shown it to be a reaction product between the cyclopropene ring of the CPFA and the sulfur of the Halphen reagents. Positive Halphen tests have been shown with other oils containing sterculic and malvalic acids; modifications of the Halphen reaction have been widely applied for qualitative tests for the presence of the

cyclopropene ring. Recently, Bailey et al. (4) modified the procedure so that quantitative data could be obtained. This method was found to be applicable to the diet and tissue lipids and was used throughout this thesis research to measure CPFA. In addition to different modifications of the Halphen test (28), other available procedures include the Durbetaki HBr titration (12, 21, 22), various methods based on GLC of the esters of lipid samples before and after hydrogenation (8, 15, 17, 58), GLC analysis of CPFA derivatives (27, 40), and infrared absorption analysis (5, 16, 32). Nuclear magnetic resonance, used in the elucidation of the structure of sterculic acid, has been proposed for its quantitative determination (44).

EXPERIMENTAL

Feeding Studies

Experimental Animals

For all feeding trials in this work, rainbow trout (Salmo gairdnerii) were used. Eggs, artificially spawned, were hatched and the fry immediately placed on experimental rations. The fish were held in four-foot circular fiberglass tanks in well water with a constant temperature of 11.4°C at a flow rate of approximately 5 gal/min. When small, the fish were fed six times daily ad libitum; when larger, feedings were once or twice a day at about 90 percent of the ad libitum amount.

Experimental Diets

Experiment I. One hundred and fifty, 4-month old fingerlings were removed from basal diet I-A (see Table 1 for composition of diets) and divided into two groups. Group A continued on the control diet (I-A), while Group B received the same diet with the addition of 400 ppm Sterculia foetida oil (I-B). (One ppm S. foetida oil is equivalent to 0.558 ppm CPFA. Therefore, I-B fish received 223 ppm CPFA.) Random samples of 12 fish each were taken from both diets at weekly intervals beginning with the second week and

Table 1. Composition of basal and experimental diets.

Diet	I-A	I-B	II-A	II-B	III-A	III-B	III-C
Corn oil	5.0	5.0	0.4	---	14.0	14.0	12.7
Salmon oil[1/]	5.0	5.0	---	---	---	---	---
S. foetida oil[2/]	---	0.04	---	0.4	---	0.04	---
Tristearin	---	---	5.0	5.0	---	---	---
Vitamin-free casein	49.4	49.4	49.4	49.4	49.4	49.4	37.5
Dextrin	15.6	15.6	15.6	15.6	15.6	15.6	10.0
Alpha-cellulose	4.9	4.9	9.5	9.5	0.9	0.9	1.4
Gelatin	8.7	8.7	8.7	8.7	8.7	8.7	8.6
Salt mixture No. 2 USP XIII	4.0	4.0	4.0	4.0	4.0	4.0	2.7
Guar gum	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Carboxymethylcellulose	1.3	1.3	1.3	1.3	1.3	1.3	1.3
70% choline chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$CaCO_3$	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Vitamin E - 50,000 IU/lb.	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Vitamin A - 250,000 IU/g	0.0865	0.0865	0.0865	0.0865	0.0865	0.0865	0.0865
Cottonseed flour[3/]	---	---	---	---	---	---	19.7
Vitamin supplement[4/]	2.0	2.0	2.0	2.0	2.0	2.0	2.0

1/ Commercial salmon oil. Donated by Bumblebee Packing Co., Astoria, Oregon.

2/ Hexane extract from *S. foetida* seeds.

3/ Proflo. Traders Oil Mill Co., Fort Worth, Texas.

4/ Vitamin supplement - percent in diet: Thiamine (HCl), 0.0064; Riboflavin, 0.0144; Niacinamide, 0.0512; Biotin, 0.00016; Ca-pantothenate (D), 0.0288; Pyridoxine (HCl), 0.0048; Folic acid, 0.00192; Menadione, 0.0016; B_{12} (cobalamine 3000 ug/g), 0.005334; i-inositol (meso), 0.2500; Ascorbic acid, 0.1200; p-aminobenzoic acid, 0.0400; D_2 (500,000 usp/g), 0.0008; Butylated-hydroxyanisole, 0.0015; Butylatedhydroxytoluene, 0.0015; Celite, 1.471586.

continuing through the sixth week of feeding. At each sampling, the fish were killed by exposure to MS 222 tricaine methane sulfonate (an anesthetic for cold blooded animals); their gastrointestinal tracts were removed to eliminate interference of undigested diet, wet weights were taken and the fish were stored at 0°F until analyzed.

Experiment II. Eight, 17-month old trout were removed from basal diet I-A and divided into two groups. Group A was placed on a control diet (II-A) with tristearin as the sole lipid source. Group B received the same diet with the addition of 4000 ppm S. foetida oil (2233 ppm CPFA; diet II-B). Corn oil was added to diet II-A at the same level (0.4%) to compensate for the additional triglycerides added to diet II-B in the S. foetida oil. After eight weeks on these diets, the fish were killed and stored at 0°F for cyclopropene and fatty acid analysis.

Experiment III. Ten fingerling trout were placed on control diet III-A with corn oil comprising the lipid portion. Ten also were fed the same diet with the addition of 400 ppm S. foetida oil (223 ppm CPFA; diet III-B). Ten additional fish were fed a diet similar to the control III-A diet with the addition of 20 percent cottonseed flour (diet III-C). Adjustments were made in other components to make the diets isocaloric and isonitrogenous. This diet contained approximately 50 ppm CPFA. After 20 months, the fish were killed by a lethal dose of tricaine methane sulfonate and

stored at 0°F for analysis.

The 223 ppm level of CPFA fed to these fish (diets I-B and III-B) represents the midpoint they would receive on a diet containing 5 percent cottonseed oil (at 0.5-1% CPFA this would provide 250-500 ppm in the diet) and what they would receive from a 20 percent cottonseed flour diet (at 5% lipid with 0.5-1% CPFA this would be 50-100 ppm CPFA), levels which could reasonably be incorporated into diets. Diet II-B contained ten times this level. Diet III-C provided approximately 50 ppm CPFA. When the diets were formulated, the best available analysis techniques showed that S. foetida oil contained 50 percent CPFA. However, a newer modification, combining GLC with selective hydrogenation, allowed more careful measurement and showed the actual composition to be 55.8 percent CPFA (45). S. foetida oil was used as the source of CPFA since it contained these components at fifty times the level of those in cottonseed oil and could be used at lower concentration in the diet to achieve the same final CPFA concentration. This minimized the interference of the remainder of the fatty acids and other components in the CPFA-carrier oil (S. foetida or cottonseed) in the diets where effects of lipid components were under investigation.

The basal diets used throughout these experiments have been formulated by Sinnhuber and coworkers (57) to provide a well

balanced ration for the rearing of rainbow trout. The lipid components and the α-cellulose (inert) were adjusted to accommodate the different types and levels of lipid under investigation. The fatty acid composition of the dietary lipids (Table 2) are included to show the type of fatty acids available to each lot of fish. The two vegetable lipids are quite similar; whereas, the salmon oil provides many long chain unsaturates and is correspondingly lower in 18:2.

Tissue Analyses

Lipid Extraction

All tissue samples and ground whole fish were extracted by the technique described by Folch et al. (18). After the centrifugation step in the Folch extraction and removal of the aqueous methanol layer, the volume of the chloroform phase was noted and approximately one-fourth of this (volume measured) was pipetted into a tared flask. The solvent was removed in a rotary evaporator and the lipid was dried to constant weight at 101°C for determination of percent lipid. The remainder of the chloroform phase was dried over anhydrous sodium sulfate and evaporated to dryness in a rotary evaporator. The lipid samples were stored under nitrogen at -10°C until used for the Halphen test or for methyl ester

Table 2. Fatty acid composition of dietary lipids.[1/]

Fatty Acid	Corn Oil	Cottonseed Flour Lipid	Salmon Oil
14:0	t	1.0	4.7
16:0	6.2	27.6	17.3
16:1	t	0.8	8.4
16:2	---	---	1.2
18:0	2.1	3.3	3.8
18:1	36.8	19.2	27.7
18:2	53.9	47.6	1.5
18:3	---	0.5	2.9
18:4	---	---	1.4
20:0	1.0	---	---
20:1	---	---	5.9
20:4	---	---	1.0
20:5	---	---	5.7
22:1	---	---	6.3
22:4	---	---	2.2
22:5	---	---	1.9
22:6	---	---	6.6

1/ Fatty acid composition as weight percentage determined by triangulation of GLC chromatograms. Minor components (less than 0.5%) were disregarded.

preparation.

Moisture Determination

Moisture and volatile matter were determined on the ground samples according to A. O. C. S. Official Method Ca-2c-25 (2). All determinations were carried out in duplicate and the values reported are the means of the two analyses.

Methyl Esterification

Approximately 50 mg of each lipid sample was esterified using the boron trifluoride-methanol procedure of Morrison and Smith (35) for triglycerides. Esters were stored at -10°C under nitrogen until analyzed by GLC.

Halphen Determination of Cyclopropene Function

The quantitative modification of the Halphen test by Bailey et al. (4) was used for both the dietary lipids and those from the fish tissues. Their specially constructed reaction vessels were replaced with 25 ml low actinic volumetric flasks with perforated aluminum foil caps. All reagents were reduced by one-half to fit this smaller reaction flask. Sample sizes were adjusted to give final absorption readings in the 0.025 to 0.250 range at 495 mu. Absorbance readings were made on all samples immediately after cooling to room

temperature using the Beckman Model DU spectrophotometer. Absorption spectra were run using the Beckman Model DK-1 recording spectrophotometer from 600 mu to 400 mu to check for purity of the Halphen pigment peak. In the dietary lipid analyses, blanks were run using all reagents except the sulfur in carbon disulfide with the dietary lipid. In the fish tissue analyses, the lipid from the fish on the Group A diets (devoid of CPFA) was used for the blank. These determinations were quantitated using a standard curve prepared from serial dilutions of S. foetida oil having a known cyclopropene concentration. The CPFA concentration of the S. foetida oil was determined using GLC in conjunction with selective hydrogenation over a promoted nickel catalyst, followed by column chromatography on 25 percent silver nitrate/silicic acid (45).

The modified Halphen test for analysis of cottonseed oils (4) was found to be applicable to the fish lipid sample analysis in this research. The characteristic red pigment formed with a maximum absorption at 495 mu as reported (Figures 1 and 2). However, the peak at 540 mu, reported to be "quite intense" in the absence of morpholine when the test was run on refined cottonseed oil by Bailey's group, was only slightly evident in the fish tissue lipids even without the use of this reagent. This is probably the result of suppression by the phosphatides present in the samples, as those workers suggest in an earlier publication (3). This 540 mu peak

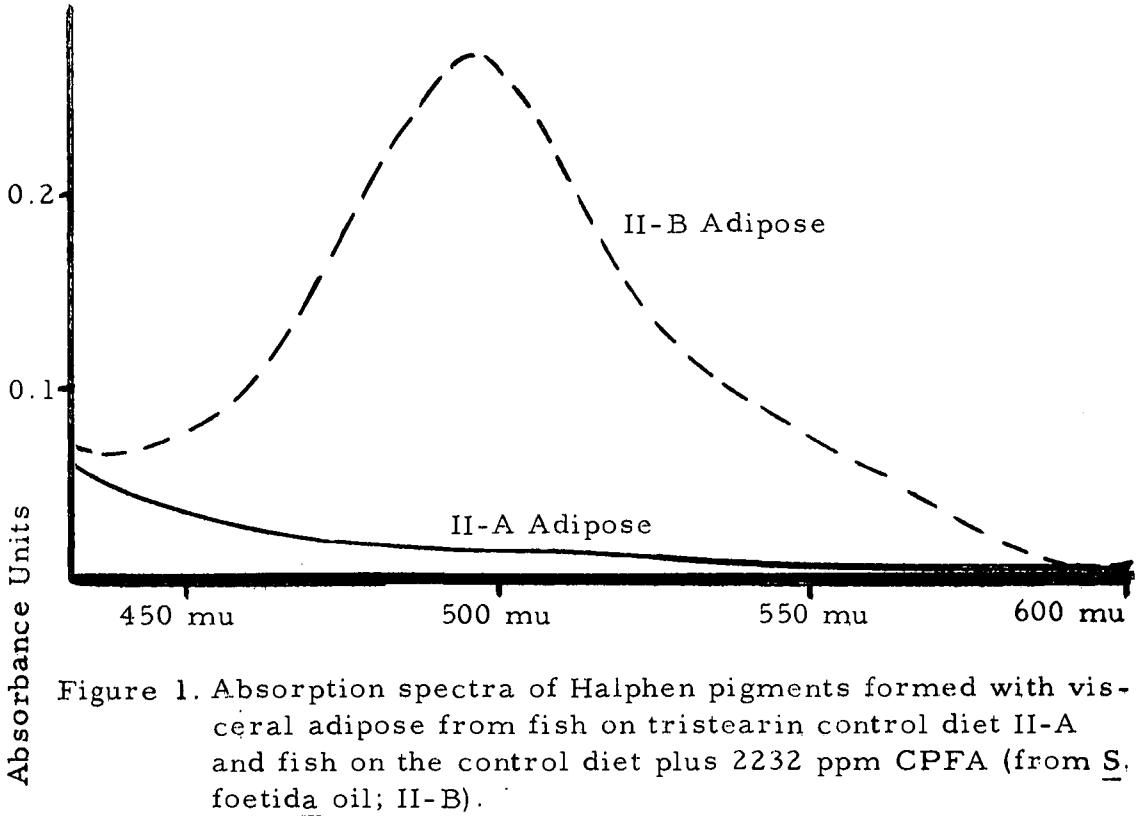


Figure 1. Absorption spectra of Halphen pigments formed with visceral adipose from fish on tristearin control diet II-A and fish on the control diet plus 2232 ppm CPFA (from S. foetida oil; II-B).

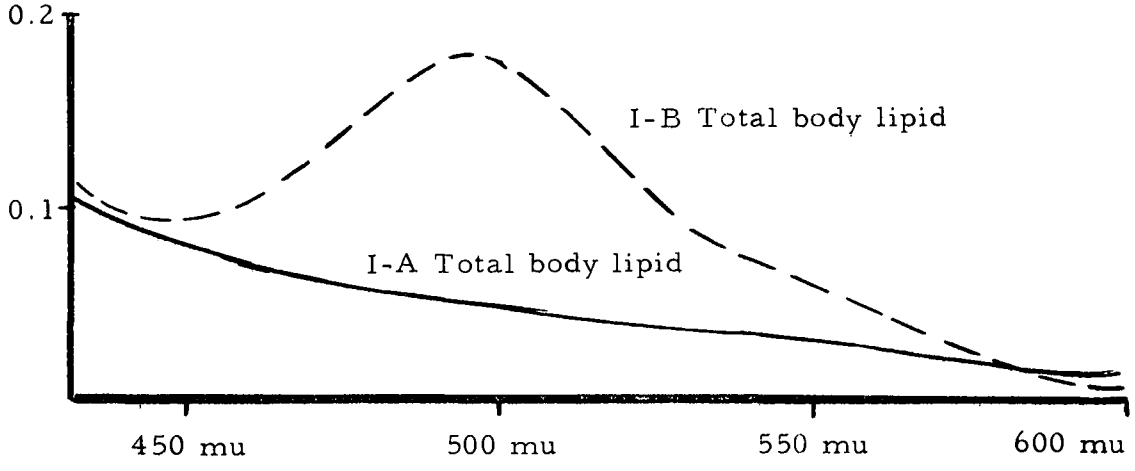


Figure 2. Absorption spectra of Halphen pigments formed with total body lipid from fish on corn oil plus salmon oil control diet I-A and fish on the control diet plus 223 ppm CPFA (from S. foetida oil; I-B).

did increase and eventually became the major peak when the reaction mixture was exposed to the fluorescent light of the laboratory. This necessitated the use of low actinic glassware and the reading of absorbance values immediately after the reaction mixture was cooled to obtain maximum accuracy and precision. (Twelve hours exposure to light reduced the absorbance values by more than 50 percent.)

Although applicable to the total body lipids and to lipids extracted from visceral adipose and belly wall muscle, this test could not be used for lipids from liver, heart, kidney and spleen. The lipids from these organs were a dark brown color and the 495 mu peak of the Halphen pigment was masked by the high non-Halphen background absorption. (Even the organs from fish on diets devoid of CPFA showed considerable absorption at 495 mu. Since these "control" organs were used for the blanks in the spectral analyses, no valid measurements of CPFA levels in the "experimental" tissues could be made.) In an effort to reduce this background absorption, methyl esters of these dark colored lipids were prepared for Halphen determinations. But these esters were as dark, if not darker, than the original lipids and consequently exhibited the same background interference. In view of these obstacles and the minor contribution of these tissue lipids to the total quantity of lipid present, no further attempts were made to measure the CPFA concentrations in the liver, heart, kidney or spleen. It is possible, however, that one

of these organs could contain a high cyclopropene concentration, thereby altering the CPFA content of the whole body lipid. This could not be demonstrated in the research of this thesis.

Gas-Liquid Chromatography

Instrument

An Aerograph Model 600-B Hi-Fi gas chromatograph with a flame ionization detector was used for analysis of methyl esters in this study. Operating parameters used were as follows:

Injection port temperature	250°C
Oven temperature	190°C
Detector temperature	250°C
Nitrogen flow rate	20 ml/min
Hydrogen flow rate	20 ml/min
Air flow rate	300 ml/min.

Column

The column used for these analyses was a 12 ft x 1/8 in O.D. aluminum column packed with 3.6 g of 10 percent C-6 diethyleneglycol succinate on 120-140 mesh Celite. Conditioning was carried out at 220°C for 48 hours prior to use.

Qualitative Analyses

Sample esters were compared with commercial standards,

when available, for identification by means of equivalent chain length (34) and/or carbon number plots (62). Purified ester standards included the following: 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:0, 20:4, 22:0, and 22:1 (Applied Science Laboratories, State College, Pa.) and 20:1, 20:5, and 22:6 (NIH Laboratories, Bethesda, Md.). For components which did not correspond to any available standards, tentative identification was made based on comparison of their equivalent chain length with published equivalent chain length values obtained from similar columns under similar conditions (23).

Quantitative Analyses

Percent composition (by weight) of the esters in the mixtures was determined by the triangulation technique (25). Quantitative results with NIH Fatty Acid Standards C and D and Applied Science Standard K-102 agree with the stated composition data with a relative error less than 5.3 percent for major components (more than 10 percent of total mixture) and less than 3.0 percent for minor components (less than 10 percent of total mixture; 24).

RESULTS AND DISCUSSION

These studies add further support to the importance of carefully inspecting natural constituents of all diets. The CPFA did have a deleterious effect on the well being of the experimental animals used in this work. The results of these studies, in general, have shown that the effects of CPFA on rainbow trout are very similar to those on other animals reported by previous investigators. The most clearly defined change, as shown in the first experiment, is the growth depression by low levels of CPFA (Figure 3). At the end of six weeks on a diet of 223 ppm CPFA (0.04% S. foetida oil) the weight of the CPFA-fed I-B fish was only 72 percent of their control I-A fish. Quantitative lipid determinations were carried out on fish from the control and test diets to investigate a histopathological observation that organs from CPFA-fed fish appeared to contain more and larger fat globules than did their controls (60). Since lipid and moisture determinations (Table 10) revealed no significant differences between fish on the test diet and the control, the apparent difference must be in the pattern of deposition rather than in the quantity of lipid deposited. This observation holds for all experiments of this thesis. Although there were no differences in the percent lipid deposited, there were significant changes in fatty acid composition of the triglycerides (Tables 3 and 4). In

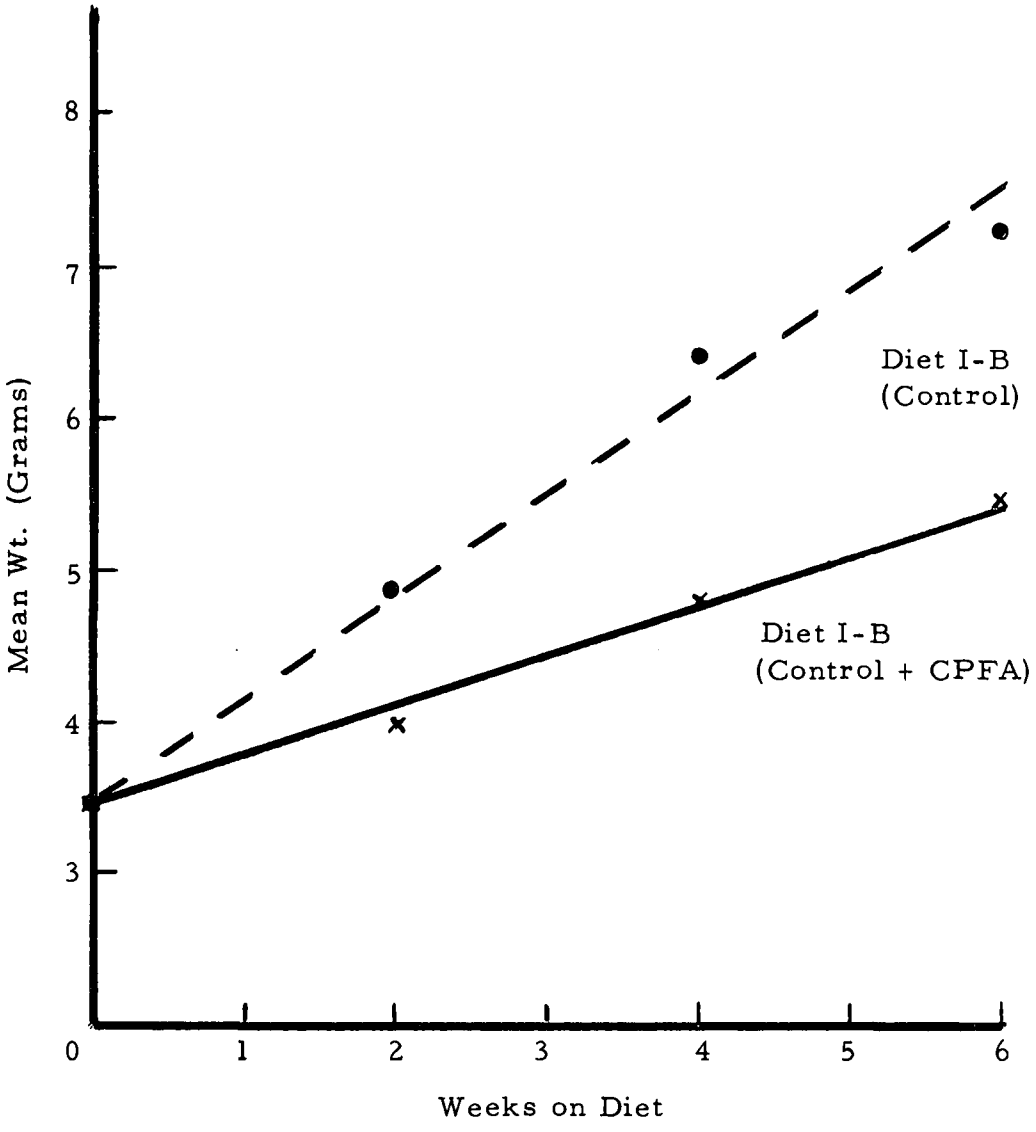


Figure 3. Growth curve of fish on corn oil plus salmon oil control diet I-A and fish on control diet plus 223 ppm CPFA (from S. foetida oil; I-B).

Table 3. Fatty acid composition of total body lipids of group I-A fish on salmon oil and corn oil control diet.[1/]

Fatty Acid	Time on Experimental Diet				
	2 Weeks	3 Weeks	4 Weeks	5 Weeks	6 Weeks
14:0	2.3	2.8	1.7	2.5	2.9
16:0	19.7	17.1	19.9	19.0	23.1
16:1	5.3	6.3	6.6	6.4	4.3
18:0	4.5	4.4	4.1	4.5	5.0
18:1	29.7	31.0	31.7	31.6	29.2
18:2	17.6	22.5	20.2	21.6	15.2
18:3	1.0	2.3	1.0	0.9	0.8
20:1	2.2	2.7	2.2	2.5	1.8
20:2 ?	3.7	1.8	1.2	1.4	2.8
20:3	1.2	1.1	1.2	0.8	1.2
20:4	0.7	0.7	0.9	0.6	1.2
20:5	2.8	0.5	0.7	0.6	1.3
22:1	0.6	1.2	1.0	1.2	1.2
22:5	1.4	0.8	1.7	0.6	1.4
22:6	5.8	3.1	4.6	4.4	6.7

1/ Fatty acid composition as weight percentage determined by triangulation of GLC chromatograms. Minor components (less than 0.5%) were disregarded.

Table 4. Fatty acid composition of total body lipids of group I-B fish on salmon oil and corn oil diet containing 223 ppm CPFA (from S. foetida oil).

Fatty Acid	2 Weeks		3 Weeks		4 Weeks		5 Weeks		6 Weeks	
	Weight %[1]	% Change[2]	Weight %	% Change	Weight %	% Change	Weight %	% Change	Weight %	% Change
14:0	2.0	-13	2.3	-18	2.5	+47	2.9	+16	2.7	- 7
16:0	21.5	+ 9	17.3	- 1	17.8	-11	18.1	- 5	22.6	- 2
16:1	4.6	-13	5.2	-17	6.0	- 9	4.8	-25	5.0	+16
18:0	5.5	+22	5.0	+14	3.4	-17	5.0	+22	5.1	+ 2
18:1	28.5	- 4	30.6	- 1	31.5	- 1	29.6	- 6	30.2	+ 3
18:2	18.1	+ 3	21.6	- 4	23.5	+16	21.8	+ 1	20.8	+37
18:3	0.6	-40	1.6	-30	1.0	0	1.3	+44	0.9	+12
20:1	2.2	0	2.8	+ 4	2.4	+ 9	2.6	+ 4	2.4	+33
20:2 ?	3.1	-16	1.6	-11	1.5	+25	3.0	+114	3.2	+14
20:3	1.2	0	1.1	0	1.1	- 8	1.0	+25	0.8	-33
20:4	0.7	0	0.7	0	0.8	-11	0.6	0	0.4	-66
20:5	1.1	-61	0.7	+40	0.3	-57	0.9	+50	0.5	-62
22:1	0.9	+50	1.1	- 8	1.4	+40	0.9	-25	1.0	-17
22:5	0.9	-36	1.2	+50	0.6	-65	0.6	0	0.5	-64
22:6	6.1	+ 5	4.6	+48	5.1	+11	4.7	+ 7	3.0	-55

1/ Fatty acid composition determined by triangulation of GLC chromatograms. Minor components (less than 0.5%) were disregarded.

2/ Percent change is the ratio of the difference in composition of I-B and the control diet (I-A) to the weight percentage in the control.

the fish tissue of CPFA diet I-B, there was an overall increase in the deposition of 18:0 and a slight decrease in 18:1, which agrees with the findings in other animals (15, 39). The relative proportion of other fatty acids deposited was also altered, but since these components are present in minor quantities, the significance of these changes is questionable. Fractionation to concentrate these minor constituents prior to GLC analysis would permit a more definite statement as to their changes. Overall, a slight increase in unsaturation appeared in the CPFA-fed I-B fish. This is evidenced by the increase in calculated iodine values (CIV; Table 10). These figures were obtained by converting the weight percentages (from the GLC data of Tables 3 and 4) to mole percentages then calculating the iodine which should be absorbed by each component considering the number of double bonds present. Summation of the iodine absorbed by each component gave the CIV. The pattern of increasing CIV continued through the first five weeks of CPFA feeding and reflected mostly the increase in 22:6 found in the total body lipid during this time. However, in the sixth week, the 22:6 component in the CPFA-fed I-B fish was reduced by more than 50 percent, with a corresponding decrease in CIV. Feeding studies should be planned to cover a longer time interval to determine if the change is magnified as feeding continues. A decrease in CIV would be expected overall in view of the results of other workers

on warm blooded animals and the results of the other parts of this thesis research. Perhaps six weeks of feeding at such a low level of CPFA were not sufficient to demonstrate this decrease.

The incorporation of CPFA into the tissue lipids by I-B fish also agrees with work on other animals where positive Halphen tests were found in tissues after ingestion of CPFA (1, 30). The quantitative Halphen test now available (4) permitted measurement of the percent CPFA in the tissues and from these values the percent incorporation could be calculated using the following equation:

$$\% \text{ Incorporation} = \frac{\text{Weight CPFA deposited}}{\text{Weight CPFA ingested}} \times 100.$$

Figure 4 illustrates the surprisingly rapid incorporation of the dietary CPFA and the high level accumulated after only six weeks on the very low dietary level. At the end of five weeks, these fish were incorporating more than two-thirds of the total CPFA fed to them. Shenstone and Vickery (53) reported deposition of approximately one-quarter of the ingested CPFA in the egg yolk lipids of hens fed sterculic and malvalic acids. Longer feeding trials enabling the fish to reach maturity are necessary to investigate the relationship of feeding time and incorporation levels in the various organs and in the eggs. The percent CPFA deposited in the fish lipid appears to be linearly related to the time on diet, or to the total CPFA consumed, throughout the entire feeding time. If this

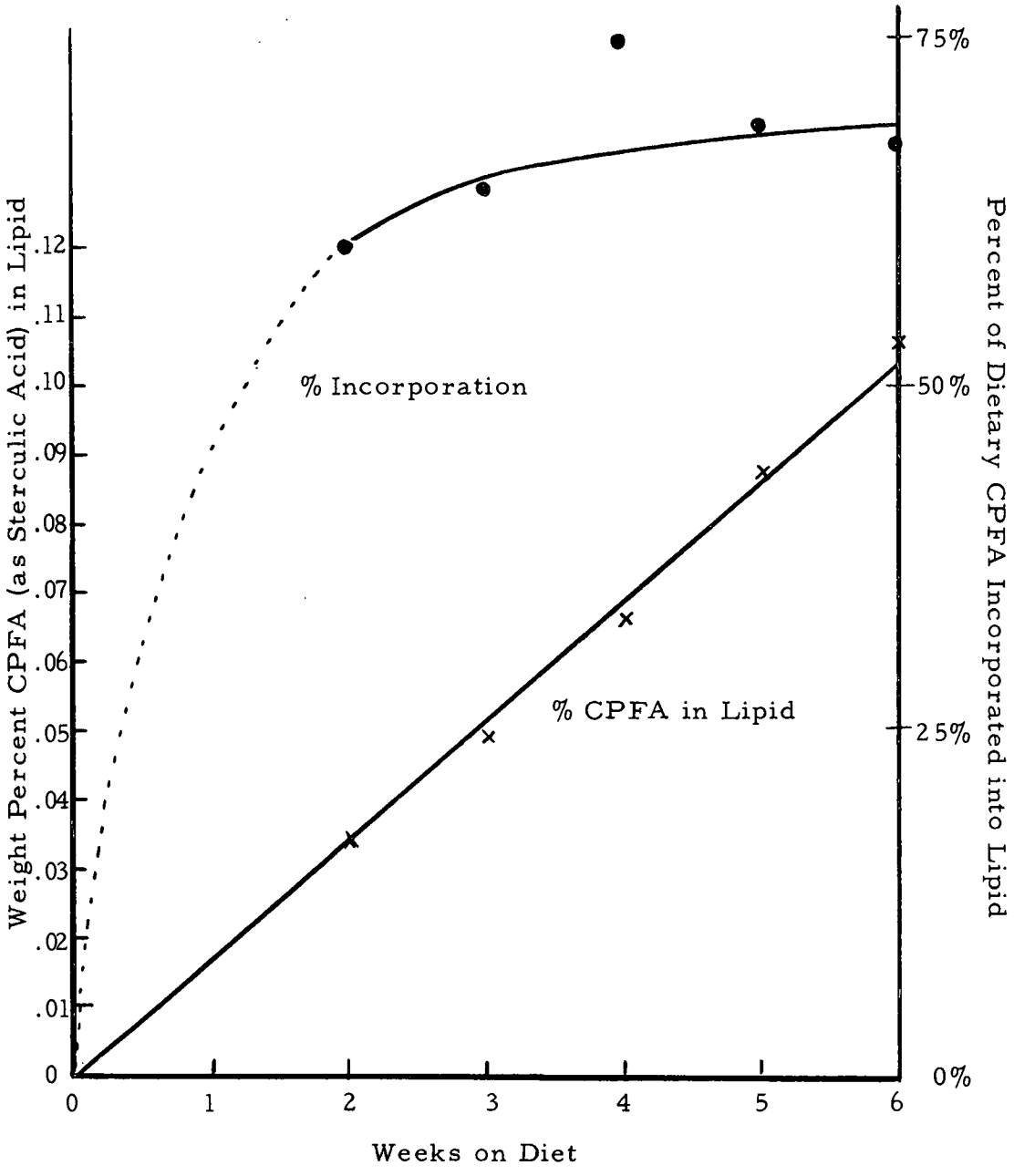


Figure 4. Incorporation of cyclopropenoid fatty acids into total body lipids of diet I-B fish on a corn oil plus salmon oil diet containing 223 ppm CPFA (from S. foetida oil).

were to continue throughout longer feeding trials, the tissue CPFA level would become unreasonably high. Since the third experiment in this thesis research demonstrated this did not occur, a longer study with more frequent samplings should be carried out to determine the break point in this curve. The question of whether the CPFA accumulate to a certain level in a linear fashion and then abruptly stop remains to be answered. From the percent incorporation curve (Figure 4), it appears that initially the fish retained a very high portion of the CPFA ingested, then reduced this retention to a nearly constant two-thirds of the intake. Many questions remain to be answered about the metabolism of CPFA. The results of this experiment show that in much of the ingested CPFA, the cyclopropene ring structure is not altered, but no information was obtained concerning changes in chain length or the extent of beta-oxidation undergone by the CPFA. In vitro studies with model systems followed by in vivo investigations of the metabolism of CPFA would provide much needed evidence for the fate of these unusual fatty acids.

Experiment II was carried out to investigate the effects of a higher level of CPFA (2232 ppm) on adult fish for a limited time on a diet containing five percent tristearin as the sole dietary lipid. These fish maintained constant weight while the experiment was under way and there were no marked differences in the percent

moisture or percent lipid of the various tissues examined (Table 10). However, there were definite changes in the fatty acid composition of all the tissues (Tables 5 and 6). As in the fish in Experiment I, and in agreement with work on other animals, there was a significant increase in 18:0 and a decrease in 18:1 in most tissues. A marked increase in 16:0 and decrease in 16:1 in all tissues and a decrease in the long chain unsaturates in most tissues led to a marked decrease in the CIV of the lipid from fish fed diet II-B. Since these fish were raised in cold water (11.4°C), such decreases in CIV, which parallel a decrease in the melting point of the tissue lipid, could conceivably hinder the mobility of the animals. (A definite increase in "firmness" of the II-B fed fish was noted at the time of dissection.) It is true that all animals in this section were on a stress diet and were forced to either utilize 18:0 or mobilize tissue lipids to provide essential fatty acids for metabolism. The lower CIV for the tristearin controls (II-A) when compared to fish on normal diets (diets containing some of the longer chain unsaturated acids) reflects a definite alteration in the pattern of lipid metabolism for all fish in this experiment. However, the CPFA-fed II-B fish felt the stress to a much greater extent. Kircher (26) reported that CPFA will react with sulfhydryl groups, and Reiser's group (42, 43) suppressed the stearate to oleate conversion in rats with CPFA and hypothesized this to be due to inhibition of fatty acid dehydrogenating

Table 5. Fatty acid composition of tissue lipids of group II-A fish on tristearin control diet. 1/

Fatty Acid	Liver	Muscle	Adipose	Kidney	Heart	Spleen
14:0	1.9	2.4	2.3	2.2	1.7	2.1
16:0	23.3	11.0	16.1	11.1	23.7	22.0
16:1	12.7	8.4	8.0	8.3	4.8	6.7
18:0	5.1	4.4	4.0	4.6	5.9	4.6
18:1	23.8	34.9	37.3	37.4	29.5	34.9
18:2	6.4	20.4	21.8	20.5	16.0	15.4
18:3	1.0	0.8	0.9	0.9	t	0.4
20:1	0.7	3.3	3.3	3.2	1.8	2.8
20:2 ?	2.0	3.1	0.9	1.4	1.0	1.6
20:3	2.6	0.9	1.0	1.2	1.5	1.1
20:4	6.0	0.7	0.8	1.8	1.6	1.4
20:5	0.6	0.7	0.5	0.7	0.6	0.4
22:1	t	1.5	1.4	1.2	0.4	1.0
22:5	1.9	0.7	t	t	0.8	t
22:6	10.0	4.8	1.4	4.2	9.3	3.8

1/ Fatty acid weight percentages determined by triangulation of GLC chromatograms. Minor components (less than 0.5%) were disregarded.

Table 6. Fatty acid composition of tissue lipids of group II-B fish on tristearin diet containing 2233 ppm CPFA (from S. foetida oil).

Fatty Acid	Liver		Muscle		Adipose		Kidney		Heart		Spleen	
	Weight %[1]	% Change[2]	Weight %	% Change	Weight %	% Change	Weight %	% Change	Weight %	% Change	Weight %	% Change
14:0	2.1	+11	2.2	- 8	2.6	+13	2.1	+ 5	2.1	+24	2.3	+15
16:0	25.4	+ 9	21.2	+93	16.9	+ 5	21.4	+90	34.2	+44	29.0	+32
16:1	4.3	-66	5.6	-33	6.6	-17	6.4	-23	3.5	-27	2.9	-57
18:0	10.1	+98	5.1	+16	4.0	0	4.4	- 4	7.7	+31	7.3	+37
18:1	30.0	+26	37.6	+ 8	36.0	- 3	34.1	- 9	23.9	-19	21.4	-39
18:2	12.5	+92	16.3	-20	20.8	- 5	16.6	-19	9.8	-39	8.2	-47
18:3	0.5	-50	0.7	-12	0.9	0	1.0	+11	t	0	0.7	+75
20:1	2.1	+57	2.9	-12	2.9	-12	1.0	-69	2.0	+ 6	2.5	-11
20:2 ?	2.4	+20	1.4	-55	0.9	0	1.9	+36	7.6	+660	12.5	+681
20:3	2.2	-15	0.9	0	0.7	-30	1.2	-17	1.4	- 7	1.0	- 9
20:4	1.0	-83	0.7	0	0.5	-38	0.9	-50	1.5	- 6	1.2	-14
20:5	0.4	-33	0.9	+29	0.4	-20	0.4	-43	t	-	0.4	0
22:1	0.6	+	1.6	+ 7	1.0	-29	1.0	-17	0.6	+50	0.6	-40
22:5	0.5	-74	t	-	t	0	t	0	t	-	t	0
22:6	4.9	-51	2.2	-54	1.8	+29	4.0	- 5	3.9	-58	2.4	-37

1/ Fatty acid composition by weight determined by triangulation of GLC chromatograms. Minor components (less than 0.5%) were disregarded. 2/ Percent change is the ratio of the difference in the weight percentage of II-B and the control diet (II-A) to the composition in the control.

enzyme(s) believed to possess thiol groups at active sites. This may be an explanation for the phenomena observed in Experiment II.

This experiment revealed some differences in responses of the various tissues to ingested CPFA. For instance, the component tentatively identified as 20:2 increased more than 600 percent in the heart and spleen lipid while in the others the change was from +36 percent in the kidney to -55 percent in the muscle lipid. Also, with respect to individual fatty acids, some tissues responded in an opposite manner than the others analyzed: 18:2 increased in the liver lipid but decreased in the other five tissues; 22:6 increased in the adipose and decreased in the remainder; 14:0 decreased in the muscle lipid but increased elsewhere. The results of these experiments do not provide sufficient evidence to account for such tissue differences.

In Experiment II, the CPFA levels were measured in the muscle and visceral adipose in the pooled II-B fish. In the muscle, 0.112 percent of the lipid was CPFA and in the adipose 0.161 percent CPFA calculated as sterculic acid. One of the females of the II-B group had a very immature skein of eggs, which showed a lipid level of 0.569 percent CPFA. Since none of the other fish contained eggs, duplicate analyses could not be made. However, this level is approximately five times that in the muscle and adipose, suggesting selective deposition of the CPFA in the egg lipid. If this finding

should be substantiated by a study involving more mature animals, it would be a very interesting comparison with other species studied. It is interesting to note that these II-B fish, on a CPFA level of ten times that of the I-B fish, deposited only slightly more CPFA in their tissue lipids. The values cannot be directly compared, however, for several reasons: 1) Differences in age and rate of growth in the test animals for the two groups, 2) differences in control diets, and 3) differences in tissues analyzed. The small I-B fish were analyzed only as "whole" fish because their organs were too small to treat individually while in II-B fish the larger size permitted analysis of individual organs. As previously explained, the liver, heart, kidney and spleen contributed such high non-Halphen background interference that their CPFA values could not be determined by the available techniques, and they also interfered in a total body Halphen test of the larger fish. Therefore, Halphen determinations could be run only on the muscle, adipose and egg lipids. It may not be unreasonable to compare the "total body" lipid of the small Experiment I fish with the "muscle" sample of the adult Experiment II fish since in these animals muscle lipid contributes the majority of the total body lipid.

Experiment III was carried out to reveal the long term effects of CPFA on lipid metabolism, which was shown to be altered during the short-term studies of Experiments I and II. As in the previous

feeding trials, there was no consistent variation in the percent moisture or percent lipid in the various tissues analyzed. The CPFA-fed III-B fish did show some differences in fatty acid deposition from their III-A controls. As in previous experiments, CPFA in the diet increased the deposition of 18:0 overall and decreased the 18:1 component. The same pattern followed for the 16-carbon fatty acids, with the mono-unsaturated acid decreasing and the saturated one increasing overall. In some tissues, both the 18:0 and the 18:1 components (or 16:0 and 16:1) decreased in percentage, but the unsaturated acid in these instances dropped more than the saturated one in relation to the control. Several of the long chain unsaturates demonstrated consistent changes, with general increases in 20:2, 20:3 and 22:5 in the CPFA-fed III-B fish lipids. Perhaps this was an attempt to compensate for the increase in saturated fatty acids and resulted in an overall increase in unsaturation as demonstrated by the higher CIV (Table 10). This was opposite to the effects shown in Experiments I and II. The difference could have been due to differences in length of time on diet, differences in basal diets or to other factors. However, it remains that the CIV did increase in the CPFA-fed III-B fish and this differs from the results found in other animals. Further work is necessary to investigate this aspect of the influence of CPFA on fatty acid deposition in rainbow trout.

The third group of fish in Experiment III on the long-term study were given their CPFA in the form of cottonseed flour (III-C) at a level of approximately 20 percent in the dry ration. This is a commercial product used for human food. The flour is low in lipid (5 percent) but contains 0. 5 percent CPFA in the lipid portion, most of which is malvalic acid. The overall CPFA level in diet III-C was 56 ppm. The reactions of these fish on the intermediate level were expected to fall between their controls without CPFA (III-A) and the higher CPFA level (III-B), but, as can be seen from Tables 7, 8 and 9, this was not the case in the tissues examined. In five out of the six tissues, the 18:0 increased and the 18:1 decreased, as would be expected on the CPFA diet. However, there was a slight decrease in the 16:0 percentages with relatively little change in the 16:1 component. As in the higher CPFA-level III-B fish, there was a consistent increase in 20:2, 20:3, 20:4 and 22:5, which were responsible for the higher CIV (Table 10) of most tissues when compared to the control III-A tissues. Neither the CIV nor the percentages for the fatty acid compositions fell consistently between the control III-A and the higher level of CPFA III-B. From this, it would appear that some factor other than the level of CPFA had an influence on lipid metabolism in these fish.

Quantitative Halphen tests were carried out on the muscle and adipose tissue of the CPFA fed fish in Experiment III. The levels

Table 7. Fatty acid composition of tissue lipids of group III-A fish on corn oil control diet. 1/

Fatty Acid	Liver	Muscle	Adipose	Kidney	Heart	Spleen
14:0	0.5	1.1	1.0	0.9	0.7	0.6
16:0	16.8	15.1	13.3	12.4	20.6	18.3
16:1	1.4	2.8	2.2	2.1	1.8	1.6
18:0	8.7	3.6	3.4	4.0	5.4	5.7
18:1	16.3	28.7	29.5	26.9	24.8	21.9
18:2	20.0	36.4	39.4	33.8	28.7	30.4
18:3	1.4	1.7	2.3	2.0	1.2	1.6
20:0	t	t	t	t	t	t
20:1	1.1	1.9	1.5	1.4	0.9	1.2
20:2 ?	3.0	2.0	2.1	1.8	1.6	2.1
20:3	3.0	1.3	1.7	1.5	1.6	1.2
20:4	10.8	1.7	1.2	3.1	4.1	3.3
22:4	1.5	t	t	t	t	t
22:5	10.0	1.3	1.1	2.4	5.1	3.2
22:6	4.4	1.3	t	1.0	1.7	0.9

1/ Fatty acid weight percentages determined by triangulation of GLC chromatograms. Minor components (less than 0.5%) were disregarded.

Table 8. Fatty acid composition of tissue lipids of group III-B fish on corn oil diet containing 223 ppm CPFA (from S. foetida oil).

Fatty Acid	Liver		Muscle		Adipose		Kidney		Heart		Spleen	
	Weight[1] %	% Change[2]	Weight %	% Change	Weight %	% Change	Weight %	% Change	Weight %	% Change	Weight %	% Change
14:0	0.7	+40	0.9	-18	0.8	-20	1.3	+44	1.4	+100	0.8	+33
16:0	20.1	+20	14.7	- 3	10.5	- 6	16.3	+31	18.7	- 9	19.3	+ 5
16:1	0.8	-43	1.5	-46	0.9	-59	0.6	-71	1.0	-44	0.9	-44
18:0	9.8	+13	3.9	+ 8	4.9	+44	6.6	+65	5.5	+ 2	7.7	+35
18:1	13.5	-17	20.2	-30	27.4	- 7	18.1	-32	18.7	-25	21.3	- 3
18:2	21.0	+ 5	47.1	+29	45.1	+14	28.0	-17	26.8	- 7	28.6	- 6
18:3	0.8	-43	2.0	+18	2.0	-13	1.1	-45	1.6	+33	1.2	-25
20:1	0.7	-36	1.0	+47	0.7	-89	0.6	-57	0.5	-44	0.7	-42
20:2 ?	3.9	+30	2.2	+10	2.5	+19	3.9	+117	1.9	+12	2.5	+19
20:3	4.6	+53	2.1	+62	2.2	+24	4.3	+133	3.2	+48	2.7	+125
20:4	8.9	-16	1.3	-18	1.0	-10	6.8	+119	7.1	+97	5.7	+73
22:4	0.8	-47	t	0	t	0	0.9	+	0.8	+	t	0
22:5	11.2	+12	1.6	+23	0.8	-19	7.8	+350	9.0	+64	5.2	+94
22:6	2.4	-45	1.0	-15	t	0	1.7	+67	1.6	- 6	2.1	+133

1/ Fatty acid composition determined by triangulation of GLC chromatograms. Minor components (less than 0.5%) were disregarded.

2/ Percent change is the ratio of the difference in the weight percentage of III-B and the control diet (III-A) to the composition in the control.

Table 9. Fatty acid composition of tissue lipids of group III-C fish on corn oil diet containing 56 ppm CPFA (from cottonseed flour). 1/

Fatty Acid	Liver	Muscle	Adipose	Kidney	Heart	Spleen
14:0	0.4	0.7	0.6	0.8	0.6	0.7
16:0	16.6	11.8	13.2	11.7	19.7	18.0
16:1	1.0	1.7	1.4	1.5	1.5	2.0
18:0	10.1	4.7	4.3	3.3	8.0	8.9
18:1	12.4	25.3	26.4	26.2	21.8	26.6
18:2	16.4	41.3	41.7	38.6	25.2	28.0
18:3	1.5	2.5	1.7	2.1	1.1	1.3
20:0	1.0	0.7	0.7	0.7	0.6	0.7
20:1	1.3	1.0	0.9	0.9	0.9	1.0
20:2 ?	4.1	2.7	2.1	2.1	2.5	2.4
20:3	4.3	2.5	2.1	2.1	3.3	2.2
20:4	14.2	2.2	2.1	2.1	3.1	4.0
22:4	1.3	t	t	t	1.3	t
22:5	13.3	2.7	1.6	1.6	6.4	3.7
22:6	2.8	t	t	t	t	0.6

1/ Fatty acid weight percentages determined by triangulation of GLC chromatograms. Minor components (less than 0.5%) were disregarded.

Table 10. Percent moisture, percent lipid and calculated iodine values for fish tissues of Experiments I, II and III.

Diet	Sample	% Moisture	% Lipid (wet wt)	Calculated Iodine Value
I. Total body analysis for Experiment I-A fish on a corn oil plus salmon oil control diet and for I-B fish on the control diet plus 223 ppm CPFA (from S. foetida oil).				
I-A	2 weeks	74.2	6.3	108
	3 weeks	75.2	6.7	106
	4 weeks	75.2	6.3	111
	5 weeks	75.5	6.0	105
	6 weeks	75.6	5.3	112
I-B	2 weeks	74.9	6.2	110
	3 weeks	75.6	6.4	110
	4 weeks	75.5	6.9	112
	5 weeks	75.1	5.6	108
	6 weeks	76.2	4.8	96
II. Tissue analysis for Experiment II-A fish on a tristearin control diet and for II-B fish on the control diet plus 2232 ppm CPFA (from S. foetida oil).				
II-A	Liver	74.8	8.0	126
	Muscle	74.1	4.6	114
	Adipose	18.9	65.5	97
	Kidney	*	*	113
	Heart	*	*	110
	Spleen	*	*	94
II-B	Liver	75.3	8.3	92
	Muscle	73.6	4.3	90
	Adipose	12.2	72.0	95
	Kidney	*	*	95
	Heart	*	*	84
	Spleen	*	*	85

Table 10. Continued

Diet	Sample	% Moisture	% Lipid (wet wt)	Calculated Iodine Value
III.	Tissue analysis for Experiment III-A fish on a corn oil control diet, for III-B fish on the control diet plus 223 ppm CPFA (from S. foetida oil), and for III-C fish on the control diet plus 56 ppm CPFA (from cottonseed flour).			
III-A	Liver	76.1	5.9	149
	Muscle	71.6	10.8	120
	Adipose	10.2	74.2	124
	Kidney	79.9	4.5	113
	Heart	79.3	3.7	123
	Spleen	78.8	4.0	112
III-B	Liver	72.9	7.5	152
	Muscle	75.0	9.0	131
	Adipose	6.2	86.9	125
	Kidney	79.4	3.5	136
	Heart	80.1	2.4	145
	Spleen	73.9	6.1	121
III-C	Liver	79.9	3.2	168
	Muscle	69.6	11.3	132
	Adipose	7.9	79.4	124
	Kidney	76.7	3.7	120
	Heart	76.3	2.9	117
	Spleen	77.1	6.5	117

* Insufficient sample available.

found were: III-B muscle, 0.159%; adipose, 0.046%; III-C muscle, 0.040%; adipose, 0.021%. The low tissue CPFA levels in the III-B fish support the earlier hypothesis that the percent CPFA in the fish lipid is not a linear function over extended feeding although it was found to exist in the six-week CPFA feeding (I-B).

It should be pointed out that unlike the tristearin plus CPFA fed fish of Experiment II, both of these Experiment III corn oil plus CPFA fed groups had higher muscle CPFA values than adipose levels. The data available do not permit an explanation of this. In view of the very different experimental parameters of the two experiments, failure to produce identical results is not surprising.

It is interesting to note that the fish on diet III-C consumed approximately one-quarter as much CPFA as the fish on diet III-B and correspondingly deposited approximately one-quarter the level in their muscle. The deposition of one-half the level in the adipose cannot be explained with the present data.

The effects of CPFA on the fatty acid composition of the tissues of the rainbow trout used in all parts of this research were great. Only the changes which were large and/or consistent were discussed previously. It should be mentioned, however, that many other changes occurred. One of these is the marked increase in a fatty acid tentatively identified as 20:2. This increase was evident in the total body lipid of fish after four weeks on diet I-B.

Also, it was found in the liver, kidney, heart and spleen lipids of the fish on diet II-B for eight weeks. Every tissue examined from the fish which had been on diet III-B for 20 months showed the change. Examples of other such changes may be seen from the data in Tables 3-9. Unfortunately, with the available data it is nearly impossible to draw any conclusions about these changes in minor components. Further studies are necessary to provide much needed information on the effects of CPFA on the metabolism of rainbow trout.

CONCLUSIONS

1. The cyclopropene ring of the cyclopropenoid fatty acids studied is relatively stable in vivo. Compounds containing the intact ring can be recovered from the tissue lipids of rainbow trout at approximately two-thirds of the level fed. In adult female fish, there appears to be a concentration of these acids in the egg lipids.

2. The cyclopropenoid fatty acids can reduce growth of rainbow trout by as much as 50 percent over their non-cyclopropene fed controls in six weeks feeding at a level of 223 ppm.

3. The cyclopropenoid fatty acids studied can alter lipid metabolism in rainbow trout.

   a. In the tissue lipids of the cyclopropene fed animals, there is a general increase in 18:0 and 16:0 with a corresponding decrease in 18:1 and 16:1 on all diets studied.

   b. On diets containing corn oil or corn oil plus salmon oil, fish fed cyclopropenoid fatty acids tended to deposit more long chain unsaturated fatty acids than did their controls. However, when provided with tristearin as the sole dietary lipid, the fish fed these compounds reduced the level of unsaturation of their tissue lipids.

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