Growth of *Tilapia Aurea* as a Function of Degree of Dietary Lipid Saturation

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Abstract: Tilapia aurea fingerlings were fed semipurified diets differing only in the degree of saturation in the lipid component (soybean oil). Growth improved significantly as degree of unsaturation in dietary lipid increased. All fish became depleted in high (> 18 carbon atom) molecular weight polyunsaturated fatty acids during the course of the study. This may indicate that desaturation and elongation of such dietary fatty acids as linoleic and linolenic acid either did not occur or was so slow that such fatty acids as 20:5n-3, 22:5n-3 and 22:6n-3 were metabolized before appearing in the depot lipids of the fish.

Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 36:172–181

The role of saturated lipids in fish diets is not clearly understood. A requirement for linolenic acid (n-3) family fatty acids has been demonstrated for rainbow trout (*Salmo gairdneri*) (Castell et al., 1972a, 1972b, 1972c), and Csengeri et al. (1979) indicated that common carp (*Cyprinus carpio*) have a requirement for n-3 fatty acids in the amount of 1.5% of the diet. Cowey and Sargent (1977) concluded from a review of the literature on fish lipid nutrition that n-3 fatty acids have essential activity for fish.

Studies conducted with channel catfish have demonstrated that diets containing beef tallow and fish oil lead to better growth than diets containing vegetable oils (Stickney and Andrews 1971, 1972; Yingst and Stickney 1979; Gatlin and Stickney 1982). The poor growth which has been found in channel catfish fed semipurified diets containing vegetable oil has been attributed to the presence of low levels of essential fatty acids. However, the fact that catfish fed animal fat grow well in the absence of significant levels of both linolenic and linoleic (n-6) family fatty acids indicates that polyunsaturated fatty acids (PUFA) may only be required in small amounts.

Growth depression from high dietary levels of linoleic acid (18:2n-6) have been demonstrated not only for channel catfish but also for rainbow trout (Yu and Sinnhuber 1975). High levels of linolenic acid (18:3n-3) can also lead to growth depression in catfish (Stickney and Andrews 1972, Stickney and McGeachin, unpubl. data) and in *Tilapia aurea* (Stickney and McGeachin, unpubl. data).

Kanazawa et al. (1980) found that *Tilapia zillii* appeared to require linoleic acid family fatty acids in the diet at a level of approximately 1%. The requirement for linoleic acid family lipids in other tilapia species has not as yet been demonstrated.

Commercial vegetable oils may vary considerably with respect to the degree of unsaturation present in their fatty acids. The more saturated a vegetable oil sample is, the higher its melting point. Thus, some vegetable lipids are solid at room temperature, while others are liquids of various viscosities. As the melting point of the lipid decreases, the degree of unsaturation increases. The purpose of this study was to determine if the degree of saturation of soybean oil can have an effect on the growth and fatty acid composition of *T. aurea* when other aspects of the diet remain constant.

This research was supported, in part, by the Texas Agricultural Experiment Station under Project 3861 and by the United States Agency For International Development under Texas A&M Research Foundation contract 4394-3. Soybean oils utilized in the research were supplied by Riceland Foods, Stuttgart, Arkansas through the courtesy of Plant Manager Giles Colbert. Arrangements for the soybean oil and its shipment to our laboratory were made by Dr. Harry K. Dupree, U.S. Fish and Wildlife Service Fish Farming Experimental Station, Stuttgart, Arkansas. Commercial feed for the tank-reared fish was supplied by Farmland Industries, Inc., Kansas City, Missouri. The assistance and cooperation of each of these individuals, agencies and companies is gratefully acknowledged.

Methods

The research was conducted at the Aquaculture Research Center of the Texas Agricultural Experiment Station and Texas A&M University. *Tilapia* *aurea* fingerlings were stocked at 10 fish/tank in aquaria containing 30 l of water each. Four treatments were tested, and 3 replicate aquaria were established for each treatment. Placement of aquaria in rows was randomized. Initial fish weights averaged 2.8 to 3.1 g for the various treatments and did not vary significantly ($P > 0.05$) among treatments.

Water was added to each aquarium at the rate of approximately 250 ml/min, resulting in a water exchange about once every 2 hours. Water entered each aquarium as a fine spray through a plastic flow regulator and exited through a siphon tube which captured water from the bottom. An airstone was provided in each aquarium to supply supplemental aeration. Well water was utilized throughout the experiment. Daily water temperature ranges were monitored with a maximum-minimum thermometer placed in one of the aquaria. Dissolved oxygen and total ammonia levels were determined at least once weekly, while pH, nitrate, nitrite, hardness and alkalinity were determined monthly.

Fish were individually weighed at the time of initial stocking and were mass weighed every 2 weeks thereafter. Growth, food conversion ratio (g of food offered/g of weight gain) and survival were determined upon termination of the experiment.

The 4 experimental diets were identical with the exception of the soybean oil (Table 1). Each diet contained 6% soybean oil, with each oil having a different degree of saturation as indicated by iodine number. Iodine numbers were determined utilizing the AOAC (1980) method. As iodine number increases, the percentage of polyunsaturated fatty acids in the sample

Table 1. Percentage Composition of Experimental Diets Fed to *Tilapia aurea*

Ingredient	Percentage
Casein	36.18
Dextrin	30.45
Cellulose	20.57
Agar	1.00
Soybean oil[a]	6.00
Vitamins[b]	0.50
Minerals[c]	5.30

[a] Four diets were formulated utilizing soybean oil with iodine numbers of 13, 66, 105 and 124.

[b] Per kilogram of feed: vitamin A ester concentrate at 200,000 IU/g, 8250 IU; cholecalciferol (D_3), 1500 IU; vitamin E, 100 IU; menadione, 15 mg; choline bitartrate, 605 mg; nicotinic acid, 110 mg; riboflavin, 22 mg; pyridoxine hydrochloride, 40 mg; thiamin mononitrate, 40 mg; D-calcium pantothenate, 100 mg; biotin, 0.11 mg; folic acid, 10 mg; vitamin B_{12}, 40 micrograms; calcium salt of ascorbic acid, 500 mg; inositol, 110 mg.

[c] Per kg of feed: $CaHPO_4 \cdot 2H_2O$, 20.7 g; $CaCO_3$, 14.8 g; KH_2PO_4, 10 g; KCl, 1 g; NaCl, 6 g; $MnSO_4 \cdot H_2O$, 350 mg; $FeSO_4 \cdot 7H_2O$, 500 mg; $MgSO_4$, 3 g; $CuSO_4 \cdot 5H_2O$, 30 mg; KIO_3, 10 mg; $ZnCO_3$, 150 mg; CoCl, 1.7 mg; $NaMoO_4$, 8.3 mg; Na_2SeO_3, 0.2 mg; $CaCl_2$, 10 g.

increases. The iodine numbers obtained for the 4 soybean oils utilized in the present experiment were 13, 66, 105 and 124 and the data obtained are presented as functions of dietary lipid iodine number. Bomb calorimetry of the soybean oil samples showed them to contain virtually the same level of gross energy. The diets were formulated to be isocaloric (3,600 kcal/kg) and isonitrogenous (32% protein).

Dry dietary ingredients were mixed in a commercial food mixer and the soybean oil was added in liquid form. Oils with iodine numbers of 13 and 66 were solid at room temperature and required heating to liquify them prior to addition. Once the diets had been thoroughly mixed, sufficient water was added to give them the consistency of dough. Each diet was then run through a feed grinder to form pellets. The pellets, which were in the form of spaghetti, were broken up by hand and dried at room temperature with the aid of a fan. The air-dried diets were stored in a freezer in plastic bags. At approximately 2-week intervals, portions of each diet were transferred into bottles from which daily rations were obtained. The bottles were stored under refrigeration. Daily feed allowances were weighed on a top-loading balance to the nearest 0.1 g.

Fish were fed twice daily, 7 days per week for a period of 10 weeks from 4 November 1981 to 14 January 1982. Feeding rate, based on dry weight of feed, was increased from 3% of body weight daily during the first 2 weeks of the experiment to 8% during weeks 3 and 4 because slow growth was observed at the lower rate. Feeding rate was reduced to 5% ofter 6 weeks and maintained at that level until termination of the study.

Following final mass weighing, the fish from each aquarium were placed in small plastic bags and frozen for fatty acid analyses. Prior to the study the fish had been maintained indoors on a commercial catfish ration. Samples of those initial fish were collected at the beginning of the study and were subjected to fatty acid analysis as a means of determining the changes which occurred as a result of the experiment.

Analyses were conducted on slurries of whole minced, homogenized fish. Methyl esters of the lipids were prepared from chloroform:methanol extracted samples according to AOAC (1980) and injected into a gas chromatograph using the column and equipment described by Gatlin and Stickney (1982). Gas chromatography was also run on each of the dietary soybean oils and the results were compared with table values published by Ashland Chemicals, Columbus, Ohio for commercial soybean oil (referred to below as the reference oil).

Data were analyzed with the Statistical Analysis System, SAS-79 (Helwig and Council 1979) using the General Linear Models procedure and Duncan's Multiple Range test. Significance was evaluated at the 0.05 level.

Results and Discussion

Water quality was within the range considered suitable for tilapia growth and survival throughout the experiment. Water temperature ranged from 27 to 30 C with diurnal fluctuations never exceeding 2 C with the exception of 1 night when pump failure allowed the water to drop to 22 C. Dissolved oxygen ranged from 6.5 to 7.5 mg/l. Total ammonia ranged from 1.0 to 3.0 mg/l, most of which was present in the incoming water. The toxic, unionized portion was never more than 7.5% of the total (Emerson et al. 1975) under the temperature and pH ranges which existed during the study (27 to 30 C and 7.4 to 8.0, respectively). *Tilapia aurea* have been shown to tolerate and even adapt to much higher levels of unionized ammonia than were present during the current study (Redner and Stickney 1979).

Nitrite ranged from 0.02 to 0.19 mg/l during the experiment and nitrate was consistently at 1.5 mg/l. Alkalinity ranged from 200 to 300 mg/l, and hardness was essentially zero.

Weight gain by fish fed the 2 most highly saturated diets was significantly less than that of fish fed the other 2 diets (Table 2). The greatest weight gain occurred with fish fed the diet with the highest iodine number. The most commonly available commercial soybean oils are likely to have iodine numbers similar to those consumed by the 2 most rapidly growing groups in the present study (iodine numbers = 105 and 124). Based on the results of this study, the use of such unsaturated soybean oils will provide a source of dietary lipid which is superior to that which is more saturated.

Feed conversion ratios (FCR) were excessively high for fish on all 4 of the experimental diets (Table 2). Since various other studies in our laboratory utilizing the same species of fish and diets containing even more highly purified ingredients have often led to excellent FCR's, it appears that all of the diets fed during the present study were poorly utilized. The fish fed aggres-

Table 2. Mean Initial and Final Weights, Food Conversion Ratios and Survival Percentages of *Tilapia aurea* Fed Soybean Oil Diets

	Average Fish Weight (g)			
Diet	Initial	Final	Food Conversion Ratio	Survival (%)
Iodine # - 13	2.8A[a]	11.7B	4.2B	100A
Iodine # - 66	2.8A	10.6B	5.2A	97A
Iodine # - 105	3.1A	16.9A	3.7BC	100A
Iodine # - 124	2.9A	18.8A	3.3C	93A

[a] Values in columns followed by the same letter are not significantly different ($P > 0.05$).

sively and were not overfed. While all FCR values were excessively high, there was a tendency for reduced ratios with increasing iodine number, indicating that the more highly saturated diets were less well utilized. In that regard, the patterns in FCR followed those for growth.

Survival ranged from 93 to 100% in the 4 experimental groups and no significant differences were found (Table 2). In no case did more than 1 fish die in a given aquarium. In 1 instance death was caused by a fish jumping out.

Percentages of the major fatty acids found in the experimental diets along with reference values obtained from a feed table are presented in Table 3. Soybean oil with the iodine number of 124 was similar to the reference soybean oil.

Considerable differences were apparent among the 4 soybean oil diets, particularly with respect to fatty acids containing 18 or more carbon units. The results of hydrogenation of the diets with iodine numbers of 13 and 66 are particularly noticeable for stearic (18:0), oleic (18:1n-9), linoleic (18:2n-6) and linolenic (18:3n-3) acids. The much higher levels of stearic and oleic acids in those 2 soybean oils compared with the reference oil and the patterns established from the 2 less hydrogenated oils indicate that hydrogenation of linoleic and linolenic acids had occurred. Dietary sources of n-3 fatty acids other than linolenic acid were not present. While the essential fatty acid requirements of *T. aurea* have not been determined, animals, in general, require fatty acids from either the n-6 or the n-3 family. Significant levels of both were only present in the 2 diets with the highest iodine numbers.

Fatty acid patterns among the fish analyzed at termination of the experiment were not as striking as among the diets, but significant differences did occur (Table 4). Oleic acid levels were higher in the experimental fish than in tank-reared fish fed a commercial diet, though fish receiving the diet with iodine number 124 soybean oil had levels similar to fish at the beginning of the study. The commercial feed utilized prior to the study did not contain supplemental lipid but did have 47.5% soybean meal in the formula along with 30% corn. Both contain some lipid, which is similar in quality. The general similarities between the fatty acid patterns in fish fed soybean oil with iodine number 124 and those fed the commercial diet held for the polyunsaturated fatty acids 18:2n-6 and 18:3n-3. Fish on the diet with iodine number 105 were similar to fish fed the commercial diet with respect to 18:2n-6 and 20:1n-9.

With the exception of a low level of 22:5n-3 detected in fish fed the iodine number 124 diet, that fatty acid and 22:6n-3 were below detection limits in fish reared on the 4 experimental diets. With the exception of fish fed the most highly saturated diet, 20:5n-3 was not detected in the experimental fish. All 3 20 and 22 carbon n-3 family fatty acids were present in

Table 3. Fatty Acid Patterns in Soybean Oil Diets and a Reference Diet[a]

Diet	Fatty Acid (Percent of Total Lipid)[b]									
	12:0[c]	14:0	16:0	16:1n-7	18:0	18:1n-9	18:2n-6	18:3n-3	20:1n-9	20:5n-3
Iodine = 13	0.3A[d]	0.2A	10.9AB	$<$ 0.1A	30.3A	54.1A	3.2C	$<$ 0.1C	nd[e]	nd
Iodine = 66	$<$ 0.1B	0.6A	10.4B	$<$ 0.1A	14.1B	72.7B	2.0C	$<$ 0.1C	nd	nd
Iodine = 105	0.3A	0.4A	11.5A	0.1A	4.9C	50.3C	29.8B	2.3B	0.3	nd
Iodine = 124	0.1B	0.2A	11.5A	0.1A	4.7C	25.2D	51.2A	6.7A	nd	nd
Reference	nd	0.1	10.5	nd	3.2	22.3	54.5	8.3	0.9	0.2

[a] Reference values taken from a lipids table (Ashland Chemicals, Columbus, Ohio).

[b] Each fatty acid percentage for the experimental diets is based on the mean of 3 analyses.

[c] The number before the colon indicates the number of carbon atoms, the number immediately after the colon indicates the number of double bonds, and the last number indicates the position of the first double bond in number of carbon units from the methyl end of the molecule.

[d] Numbers in columns followed by the same letter are not significantly different at the 0.05 level.

[e] nd = not detected

Table 4. Fatty Acid Patterns in *Tilapia aurea* Fed Soybean Oil Diets and a Commercial Diet.

	Fatty Acid (Percent of Total Body Lipid)[a]											
Diet	12:0[b]	14:0	16:0	16:1n–7	18:0	18:1n–9	18:2n–6	18:3n–3	20:1n–9	20:5n–3	22:5n–3	22:6n–3
Iodine = 13	0.1A[c]	2.8AB	22.6A	8.1A	8.3A	49.0B	2.8D	0.8B	2.2A	0.2A	nd[d]	nd
Iodine = 66	0.1A	2.8AB	20.7B	7.7AB	7.3B	54.1A	2.3D	1.8AB	2.1AB	nd	nd	nd
Iodine = 105	0.5A	2.3BC	22.6A	6.1BC	7.3B	44.6C	12.4C	0.7B	1.4BC	nd	nd	nd
Iodine = 124	0.3A	2.0C	20.8B	4.8C	6.5C	33.6D	22.5A	2.3A	0.9C	nd	0.2A	nd
Commercial[e]	0.4A	3.1A	19.8C	7.4AB	7.0BC	30.2E	15.1B	1.9AB	1.7AB	0.1A	0.5A	1.1

[a] All values are based on the mean of 3 replicates.

[b] The number before the colon indicates the number of carbon atoms, the number immediately after the colon indicates the number of double bonds, and the last number indicates the position of the first double bond in number of carbon units from the methyl end of the molecule.

[c] Numbers in columns followed by the same letter are not significantly different at the 0.05 level.

[d] nd = not detected

[e] This group of fish was representative of the experimental fish at the initiation of the feeding experiment. The fish had been fed a commercial feed (Farmland Industries, Kansas City, Missouri) containing 32% protein in the form of meat and bone meal, soybean meal and corn meal.

fish fed commercial feed, but those fatty acids only appeared in small quantities. Such high molecular weight fatty acids are characteristic of fish which have been fed fish oil or fish meal diets (which contain small amounts of fish oil). The commercial diet utilized in this study contained meat and bone meal as the only animal protein source and was not supplemented with lipids.

It has been demonstrated that various fishes are able to elongate 18:3n-3 to higher molecular weight n-3 fatty acids (as reviewed by Cowey and Sargent 1977), though this may occur at a rate that is too slow to allow for significant levels of deposition in the tissues. The absence or very low levels of high molecular weight n-3 fatty acids in fish reared during the present experiment indicate that if chain elongation did occur, the fatty acids produced were rapidly utilized. Kanazawa et al. (1980) found that *Tilapia zillii* require n-6 fatty acids in their diet and that they can convert 18:2n-6 to 20:4n-6. If a similar conversion occurred in *T. aurea* during the present study, the gas chromatography did not reveal it. No fatty acids in either the n-6 or n-3 families occurred at levels above a few tenths of a percent except 18:2n-6 and 18:3n-3.

The levels of 18:2n-6 in fish fed the diets with iodine numbers of 13 and 66 appeared to reflect dietary levels in their tissue lipids (Tables 3 and 4), while fish on diets with soybean oil iodine numbers of 105 and 124 did not appear to retain 18:2n-6 in their tissues at levels proportional to those present in the diets. Even though the 2 diets with the lowest iodine numbers contained only trace amounts of linolenic acid, there was no depletion of 18:3n-3 in the fish fed those oils as compared with fish on the diets containing higher levels of linolenic acid (Tables 3 and 4). Because of the virtual absence of high molecular weight n-3 fatty acids in all of the fish, no desaturation and elongation of linolenic acid seemed to occur. This brings into question the essentiality of n-3 fatty acids in the diet of *T. aurea.*

In conclusion, it seems apparent that feed grade soybean oil will support much better growth of *T. aurea* than soybean oil which has been hydrogenated beyond the normal level. It is also clear that the fish utilized in this research reflected the fatty acid patterns of the feed only to a limited extent and that the feeding of extremely high levels of 18:2n-6 did not lead to high levels of deposition of that fatty acid in the body lipids. High molecular n-3 polyunsaturated fatty acids were depleted in all groups of fish, whether or not significant levels of 18:3n-3 were present in the diet. Because of relatively poor growth and poor food conversion efficiencies, soybean oil appears to be a generally undesirable lipid source for *T. aurea.* Further studies comparing soybean oil with other feed grade lipids will be needed if this question is to be pursued.

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