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A Comparison of Vitamin A1 & A2 Levels & the Role of Beta-Carotene & Lutein in the Synthesis of Vitamin A in Freshwater Fishes

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Western Kentucky University

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Benjamin J., Jr.

1980

A COMPARISON OF VITAMIN A_1 AND A_2 LEVELS AND THE ROLE OF BETA-CAROTENE AND LUTEIN IN THE SYNTHESIS OF VITAMIN A IN FRESHWATER FISHES

A Thesis

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Western Kentucky University

Bowling Green, Kentucky

In Partial Fulfillment

of the Requirements of the Degree

Master of Science

by

Benjamin J. Del Tito, Jr.

May 1980

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AND THE ROLE OF BETA-CAROTENE AND LUTEIN
IN THE SYNTHESIS OF VITAMIN A IN FRESHWATER FISHES

Recommended April 14, 1980
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Approved April 16, 1980
(Date)

Elmer Gray
Dean of the Graduate College

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A COMPARISON OF VITAMIN A_1 AND A_2 LEVELS
AND THE ROLE OF BETA-CAROTENE AND LUTEIN
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Benjamin J. Del Tito, Jr. May, 1980 29 pages
Directed by: Robert D. Hoyt, Frank R. Toman and Larry N. Gleason.
Department of Biology Western Kentucky University

The objectives of this study were to determine naturally occurring concentrations of vitamin A_1 and A_2 in selected native fishes and, under laboratory conditions, to determine the possible role of beta-carotene and lutein in the synthesis of vitamin A by goldfish. The native fishes were selected to exhibit different feeding habits and were taken from different habitats (stream vs. lake) during different seasons of the year. In the laboratory, two possible precursors, beta-carotene and lutein, were incorporated into the diet to determine the role of these substances in the synthesis of vitamin A.

Species of fish differed in amounts of vitamin A_1 and A_2 by their ability to metabolize vitamin A from their environment. Seasonally, vitamin A_1 and A_2 were in highest concentration during the fall.

Drake's Creek fishes had a higher concentration of vitamin A_1 and A_2 than did those from Rough River Lake; however, no significant difference was observed.

Goldfish readily converted beta-carotene and lutein to vitamin A_1 but only lutein conversion to vitamin A_2 was suggested. Vitamin A_1 conversion to A_2 was also observed in this study.

INTRODUCTION

Vitamin A was first described as a fat-soluble, growth promoting factor in rats by Hopkins (1912) and later by Osborne and Mendel (1914). It has since been shown to occur in two forms, vitamin A_1 or retinol, and vitamin A_2 or dehydroretinol. Vitamin A_1 is closely related chemically to vitamin A_2, the latter containing an extra double bond in the beta-ionine ring. Vitamin A_1 occurs in vertebrates, while vitamin A_2 is found only in freshwater fishes or in waterfowl which feed on freshwater fishes.

The need for vitamin A in fish was first reported by Halver (1957) when he observed xeropthalmia and cataracts in fish fed vitamin A deficient diets. Vitamin A is a stimulus for new cell growth and aids in maintaining resistance to infection. It increases longevity under various conditions of senility in mammals. Vitamin A and retinal (the aldehyde form of vitamin A) are essential for normal vision in vertebrates (West et al., 1966). Hypovitaminosis A in fish is characterized by poor growth, poor vision, keratinization of epithelial tissue, xeropthalmia, night blindness, hemorrhage in the anterior chamber of the eye, hemorrhage at the base of the fins, and abnormal bone formation (Halver, 1972).

Jones et al. (1966) have shown a vitamin A requirement for growth in fish held in light, but not in darkness, and Dupree (1966) reported similar results in determining requirements in catfish. Specific requirements for vitamin A have not been completely determined for fish (Halver, 1972). Aoe et al. (1968) estimated the vitamin A requirement of young carp to be 400-2,000 U.S.P. units/100g of diet.

Vitamin A levels fluctuate seasonally and interspecifically in freshwater fishes (Lovern et al., 1939; Pugsley, 1939). Morton and Creed (1939) found conversion of beta-carotene to vitamin A in three freshwater fish, as did Neilands (1947) in two marine species. Poston (1968) found brook trout unable to convert beta-carotene to vitamin A, concluding that only the vitamin source can be utilized by this species. Gross and Budowski (1966) found lutein devoid of provitamin A (precursor) properties in two freshwater species, but Barua and Das (1975) and Czeczuga and Czerpak (1976) detected conversion of lutein to vitamin A, specifically A_2, in selected freshwater species. Morton et al. (1947), Naito and Wilt (1962), and Hata et al. (1973) reported conversion of vitamin A_1 to A_2 in three freshwater species.

The objectives of this study were to determine naturally occurring concentrations of vitamin A_1 and A_2 in selected native fishes and, under laboratory conditions, to determine the possible role of beta-carotene and lutein in the synthesis of vitamin A by goldfish. The native fishes were selected

to exhibit different feeding habits and were taken from different habitats (stream vs. lake) during different seasons of the year. In the laboratory, two possible precursors, beta-carotene and lutein, were incorporated into the diet to determine the role of these substances in the synthesis of vitamin A.

STUDY AREA

Native fishes used in the study were taken from Drake's Creek, a small stream, and Rough River Lake, a small flood control lake.

Drake's Creek is a small tributary of the Barren River in south-central Kentucky. It is 39 km in length rising in Sumner County, Tennessee, and ending at its mouth in Warren County, Kentucky, 6 km east of Bowling Green. The sampling site was located on the West Fork of Drake's Creek at the bridge off Cedar Bluff Road, 11 km downstream from the Franklin, Kentucky, sewage lagoon (Figure 1).

Rough River Lake is a small impoundment in the Green River watershed in west-central Kentucky. The lake was impounded in 1961 with the construction of an earthen-fill dam at River Kilometer 143. The lake impounds 62 km of the Rough River at seasonal pool with an average surface of 2,345 ha and a total volume of 140 million m^3 of water. The lake has a drainage area of 1,180 km in Breckinridge, Grayson, and Hardin Counties. Rough River Lake fishes used for analysis were taken from the main body of the lake, just above the confluence of the North and South Forks (Figure 2).

Figure 1. Map of Drake's Creek, Kentucky, showing the location of the collecting station.

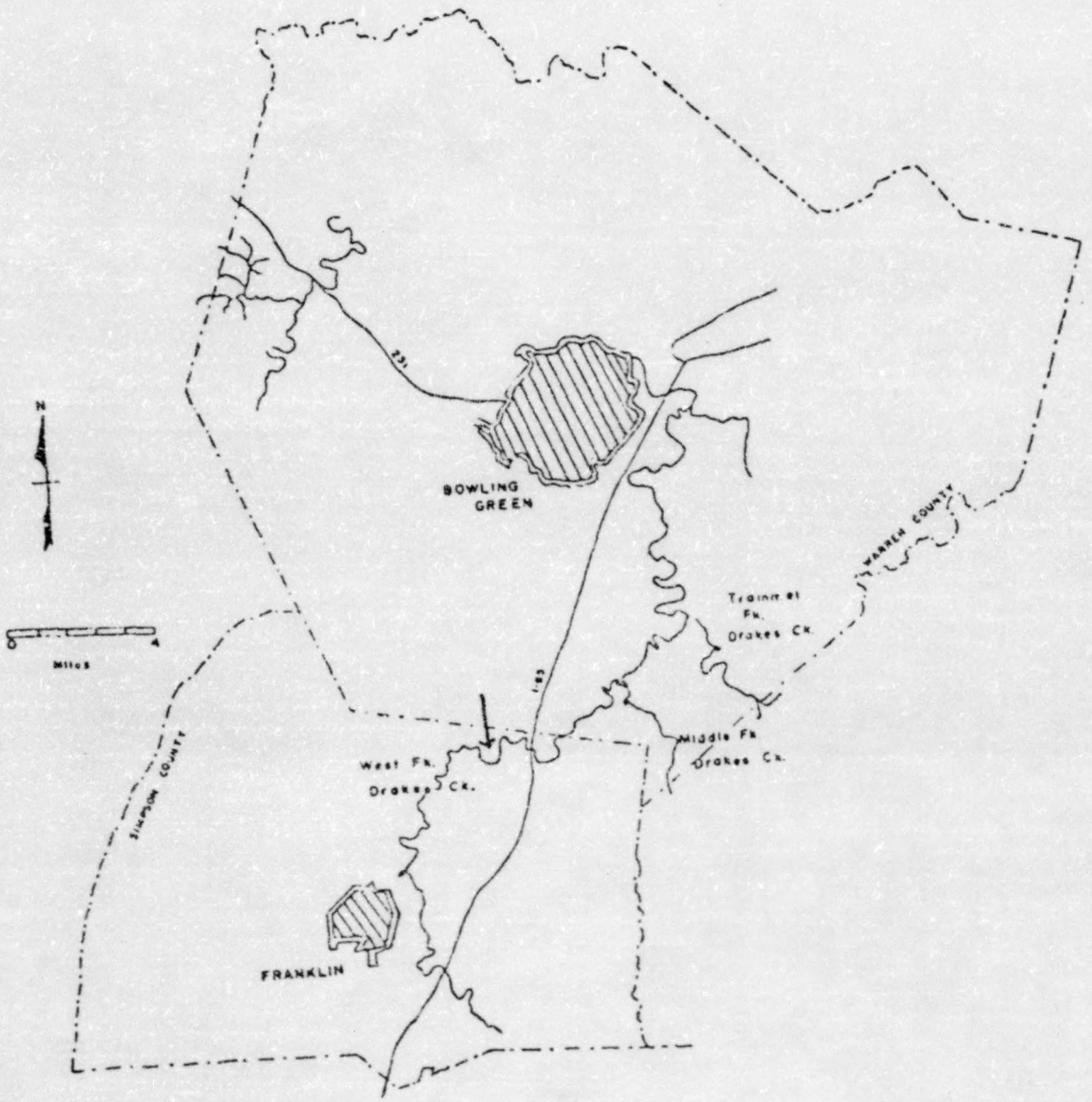
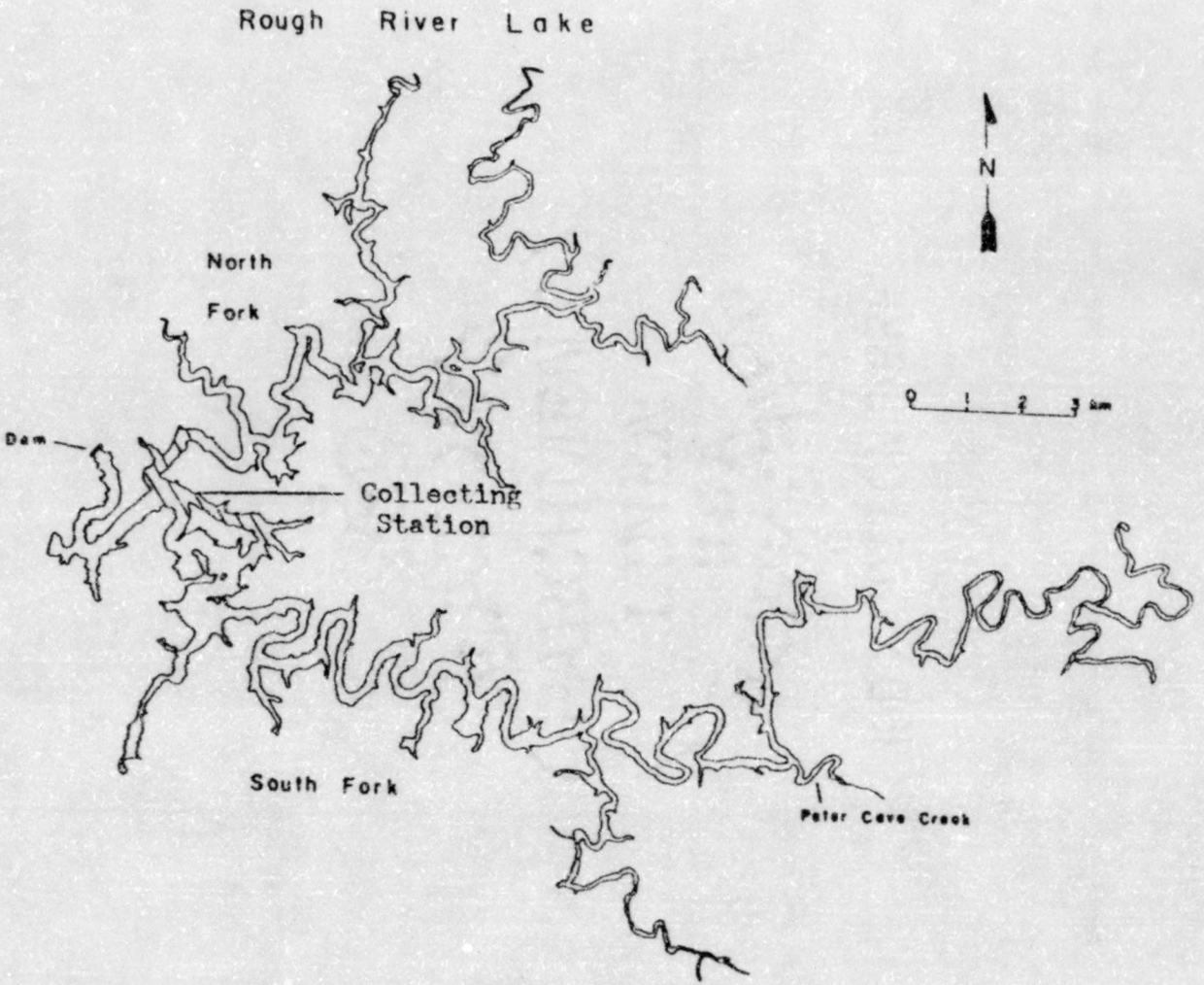

N
Miles
231
BOWLING GREEN
SIMPSON COUNTY
WARREN COUNTY
Trainor Fk. Drakes Ck.
Middle Fk. Drakes Ck.
West Fk. Drakes Ck.
FRANKLIN

Figure 2. Map of Rough River Lake, Kentucky, showing the location of the collecting station.


Rough River Lake
N
North
Fork
0 1 2 3 km
Dam
Collecting
Station
South Fork
Peter Cave Creek

METHODS AND MATERIALS

Fish samples from Drake's Creek were obtained by electrofishing techniques. Two specimens of the northern hog sucker, Hypentelium nigricans (LeSueur), and golden redhorse, Moxostoma erythrurum (Rafinesque), were collected seasonally: fall (November 2, 1978), winter (February 2 and March 2, 1979), spring (April 23, 1979), and summer (August 8 and 10, 1979). Immediately after collection, specimens were placed in ice for transport to the laboratory. In the laboratory, total weights and lengths of the fish were determined and recorded. The entire alimentary canal, excluding the liver, was excised and preserved in 2% formalin for subsequent content examination. Stomach contents were identified using keys by Pennak (1978) and Smith (1950). The livers were separated and frozen at -20 C for subsequent vitamin A analysis.

Fish samples from Rough River Lake were obtained via commercial fishermen using gill nets. Two specimens of the black buffalo, Ictiobus niger (Rafinesque), four smallmouth buffalo, Ictiobus bubalus (Rafinesque), and four flathead catfish, Pylodictis olivaris (Rafinesque) were collected seasonally: fall (November 10, 1978), winter (March 1 and 9, 1979), spring (May 9, 1979), and summer (July 24, 1979). The summer collection was obtained from a toxicant fish sample made by biologists of the Kentucky

Department of Fish and Wildlife Resources. No black buffalo were taken in the summer toxicant sample. Specimens were measured (total weights and lengths) and eviscerated in the field. The entire alimentary canal, including the liver, was placed in ice for transport to the laboratory. In the laboratory, the alimentary canals and livers were treated as above and age determinations of smallmouth buffalo made.

Goldfish, Carassius auratus (Linnaeus), 18-24 cm total length, of the wild genetic strain were obtained November 17, 1978, from the Kentucky Department of Fish and Wildlife Resources' Cave Run Fish Hatchery in Morehead, Kentucky. The fish were delivered to Western Kentucky University and placed in 570 l aquaria containing tap water, aerated to remove traces of chlorine.

The fish were held in these tanks from November 17, 1978, to February 18, 1979, to obtain acclimation and temperature control. The temperature ranged from 15 to 24 C throughout the study period with an average of 19.2 C. Illumination was provided by four overhead flourescent lamps and was restricted to a short winter day length (eight hours light, sixteen hours dark). Debris was siphoned from the tanks and fresh conditioned water replaced weekly. Water samples were analyzed for the presence of algal contamination during each cleaning.

Twenty-four goldfish were separated into 4 groups of 6 fish each. At the start of the study, November 18, 1978, two goldfish were killed and their livers frozen. These

two fish were referred to as "Initials" and were used to determine the vitamin A content present in the fish prior to the experiment. The remaining 22 fish were then fed a vitamin A deficient custom trout diet (ICN Nutritional Biochemicals) for 75 days (February 19 to May 4, 1979) to allow for the removal of all residual body vitamin A. At the end of this time period, May 4, 1979, two additional fish, Initials+75, were killed and used to determine the vitamin A content following the initial treatment. For the next 75 days, May 5 to July 18, 1979, the fish were fed four different diets. Group I (Controls) was fed a commercial grade, high protein trout chow containg 350 U.S.P. units of vitamin A_1/100g of diet (Allied Mills, Inc.). Group II (Deficients) was fed the vitamin A deficient food described for the first phase of the study. Group III (Beta-carotenes) was fed a vitamin A deficient, beta-carotene supplemented diet (104, 877 United States Pharmacopeia [U.S.P.] units/100g of diet, ICN Nutritional Biochemicals). Group IV (Luteins) was fed a vitamin A deficient, lutein supplemented diet (104, 877 U.S.P. units/100g of diet, ICN Nutritional Biochemicals). Beta-carotene was purchased from and supplemented into the diet by ICN. Lutein was extracted from egg yolk and provided to ICN for supplementation. Fish were fed 1.5% of their average total weight per day.

The lutein extraction technique used in this study was essentially that described by Horwitz (1975). The only

adjustment to this method was the use of a chromatography column containing magnesium oxide and diatomaceous earth in a 1:1 ratio using petroleum ether as a solvent. The sample was dissolved in petroleum ether and examined spectrophotometrically. The yellow product produced the three characteristic lutein peaks of 427, 445, and 475 nm described by Barua et al. (1973).

The technique for extraction of vitamin A used in this study was that described by Ames et al. (1954). Antimony trichloride and chloroform were added to the extract and it was examined spectrophotometrically for the presence of vitamin A_1 (620 nm), and A_2 (693 nm). The amounts of vitamin A_1 and A_2 present were determined by reference to a standard curve prepared with retinol (Sigma Biochemicals) and dehydroretinol (Hoffman-La Roche, Basle, Switzerland). Using the United States Pharmacopeial Convention (1955) vitamin A reference of 1 mg equaling 3,333 U.S.P. units, all quantities were converted and expressed in these units.

The experimental data were analyzed using a randomized block analysis of variance design. All calculations were made with a Texas Instrument calculator, model SR-51II. Analysis of significant F values were performed using the Student-Newman-Kuels multirange test.

RESULTS

Field Data

The northern hog sucker had the highest average vitamin A_1 levels (7,431 U.S.P. units/g of liver) followed by the black buffalo (6,142), flathead catfish (5,350), smallmouth buffalo (3,546), and golden redhorse (3,386) (Table 1; Figure 3). The vitamin A_1 levels of these species represented four statistical populations with the hog sucker, black buffalo, and flathead catfish being significantly different from each other and the smallmouth buffalo and golden redhorse being similar, but significantly lower ($P < 0.05$) than the other species (Figure 3).

Vitamin A_2 mean concentrations exhibited a similar species trend with the northern hog sucker having the highest concentration (6,406 U.S.P. units/g of liver) followed by the black buffalo (4,854), flathead catfish (4,789), smallmouth buffalo (3,013), and golden redhorse (2,325) (Figure 4). Smallmouth buffalo and golden redhorse levels were again similar, while the species with the highest concentrations showed a series of overlapping patterns of significant differences (Figure 4).

Stomach content analysis of field species exhibited no discernable differences in feeding habits. Instead, it was observed that stream and lake species fed commonly on similar organisms.

Table 1. Seasonal levels of vitamin A_1 and A_2 in two stream fishes (Drake's Creek) and three lake fishes (Rough River Lake) for 1978-1979.

Collection	Location	Species	Mean Tot. Lgth. ± SEM[1] (cm)	Mean Tot. Wt. ± SEM (g)	Mean A_1 ± SEM (U.S.P. units/ g liver)	Mean A_2 ± SEM (U.S.P. units/ g liver)
Fall	Drake's Creek n=4	Hog Sucker	28±1	208± 24	12507±4402	11141±4295
		Golden Redhorse	30±3	329±100	5374±1513	3067±1367
	Rough River Lake n=10	Black Buffalo	70±6	5619±10	13166±401	10217±1190
		Smmth. Buffalo	52±1	2384±7	6131±1733	5531±1283
		Flathead Catfish	71±4	4115±7	8182±2215	7063±1715
Winter	Drake's Creek n-4	Hog Sucker	28±0	248±27	5606±660	4651±271
		Golden Redhorse	31±4	363±98	3187±151	2866±424

[1]SEM = Standard Error of the Mean

Table 1. Continued.

Collection	Location	Species	Mean Tot. Lgth. ± SEM (cm)	Mean Tot. Wt. ± SEM (g)	Mean A_1 ± SEM[1] (U.S.P. units/ g liver)	Mean A_2 ± SEM[2] (U.S.P. units/ g liver)
Winter (continued)	Rough River Lake n=10	Black Buffalo	76±9	6016±9	3249±1072	2733±1154
		Smmth. Buffalo	53±2	2582±6	2599±448	2612±481
		Flathead Catfish	72±4	4965±9	4101±1004	3240±848
Spring	Drake's Creek n=4	Hog Sucker	24±2	154±32	6688±1635	5486±485
		Golden Redhorse	30±0	280±6	2654±127	1727±85
	Rough River Lake n=10	Black Buffalo	83±2	8981±6	3367±802	2837±1106
		Smmth. Buffalo	51±1	2206±7	2891±246	2186±351
		Flathead Catfish	36±1	467±56	4657±1148	4925±1063
Summer	Drake's Creek n=4	Hog Sucker	30±2	278±40	4924±1431	4346±612
		Golden Redhorse	28±1	196±23	2334±176	1638±192

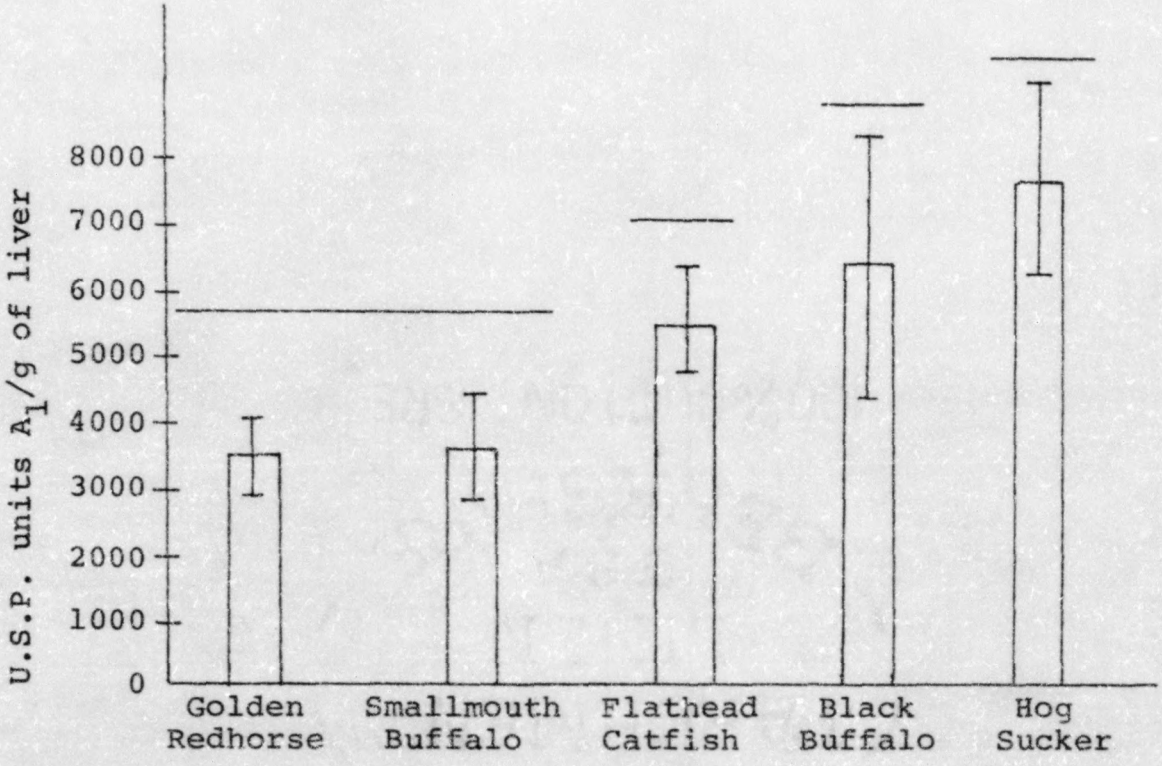
Table 1. Continued.

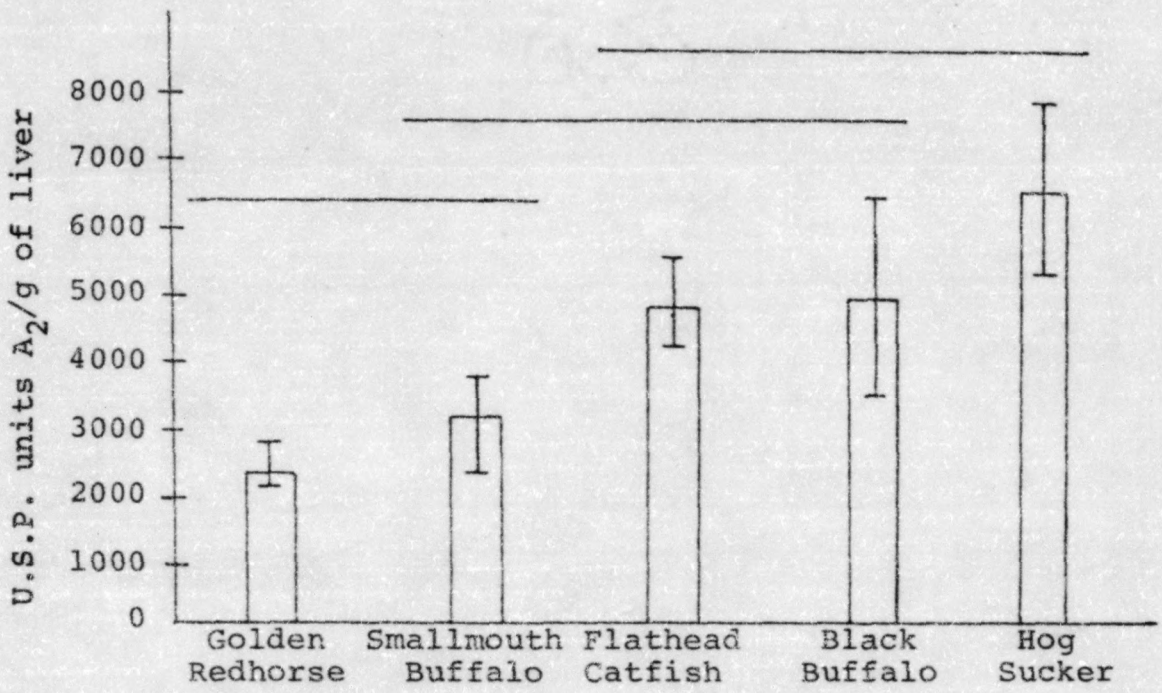
Collection	Location	Species	Mean Tot. Lgth. ± SEM (cm)	Mean Tot. Wt. ± SEM (g)	Mean A_1 ± SEM[1] (U.S.P. units/ g liver)	Mean A_2 ± SEM[2] (U.S.P. units/ g liver)
Summer (continued)	Rough River Lake n=8	Black Buffalo*	76±6	6872±10	4783±1050	3630±1232
		Smmth. Buffalo	50±1	1901±7	2562±454	1726±488
		Flathead Catfish	43±4	965±26	4458±321	3929±123
	Drake's Creek n=16	Hog Sucker	28±1	222±13	7431±865	6406±798
		Golden Redhorse	30±0	292±18	3386±343	2324±188
Grand Species Means	Rough River Lake n=40	Black Buffalo	76±1	6872±10	6142±1184	4854±899
		Smmth. Buffalo	52±0	2268±6	3546±433	3013±429
		Flathead Catfish	56±5	2628±8	5350±476	4790±417

*Mean values estimated according to Yates (1933)

Figure 3. Mean vitamin A_1 levels (U.S.P. units/g of liver) of five species of fish collected in 1978-1979. Any vitamin A_1 levels not spanned by the same line are significantly different ($P<0.05$).

Figure 4. Mean vitamin A_2 levels (U.S.P. units/g of liver) of five species of fish collected in 1978-1979. Any vitamin A_2 levels not spanned by the same line are significantly different ($P<0.05$).


U.S.P. units A_1/g of liver
8000
7000
6000
5000
4000
3000
2000
1000
0
Golden Redhorse
Smallmouth Buffalo
Flathead Catfish
Black Buffalo
Hog Sucker


U.S.P. units A_2/g of liver
8000
7000
6000
5000
4000
3000
2000
1000
0
Golden Redhorse
Smallmouth Buffalo
Flathead Catfish
Black Buffalo
Hog Sucker

Seasonally, vitamin A_1 means were in greatest concentration in the fall months (9,072 U.S.P. units/g of liver) followed by spring (4,051), summer (3,812), and winter (3,748). However, only the fall levels were significantly different, being greater than the remaining three seasons (Figure 5). A similar pattern of seasonal values was observed for vitamin A_2 concentrations. The fall level was greatest (7,403), followed by spring (3,432), winter (3,220), and summer (3,053) (Figure 6). Again, the fall value represented the only significantly different group.

Drake's Creek fishes had a higher mean concentration of vitamin A_1 (5,408 U.S.P. units/g of liver) than did Rough River Lake specimens (5,012); however, no significant difference was observed (Figure 7). A similar pattern was observed for vitamin A_2 concentrations. Drake's Creek fishes had a higher concentration (4,365) than did those from Rough River Lake (4,219), but again no significant difference was observed (Figure 8).

Age levels of smallmouth buffalo reflected no perceptible trends in vitamin A_1 or A_2 levels.

Laboratory Data

In goldfish the Beta-carotenes had the highest average vitamin A_1 levels (4,921 U.S.P. units/g of liver) followed by the Controls (4,896), Initials (3,888), Luteins (2,755), Deficients (207), and Initials+75 (37) (Table 2; Figure 9). The Deficient and Initials+75 levels were statistically

similar, being significantly lower than the other groups which showed overlapping patterns of significance ($P < 0.05$) (Figure 9).

Vitamin A_2 concentrations exhibited a somewhat different trend with the Control group having the highest concentration (4,542 U.S.P. units/g of liver) followed by the Initials (3,761), Beta-carotenes (3,427), Luteins (2,716), Deficients (400), and Initials+75 (40) (Figure 10). The experimental group vitamin A_2 levels represented two statistical populations with the Deficients and Initials+75 again being similar, and the Controls, Initials, Beta-carotenes, and Luteins being similar but significantly higher than the previous population (Figure 10).

Table 2. Vitamin A_1 and A_2 levels of goldfish by experimental treatment.

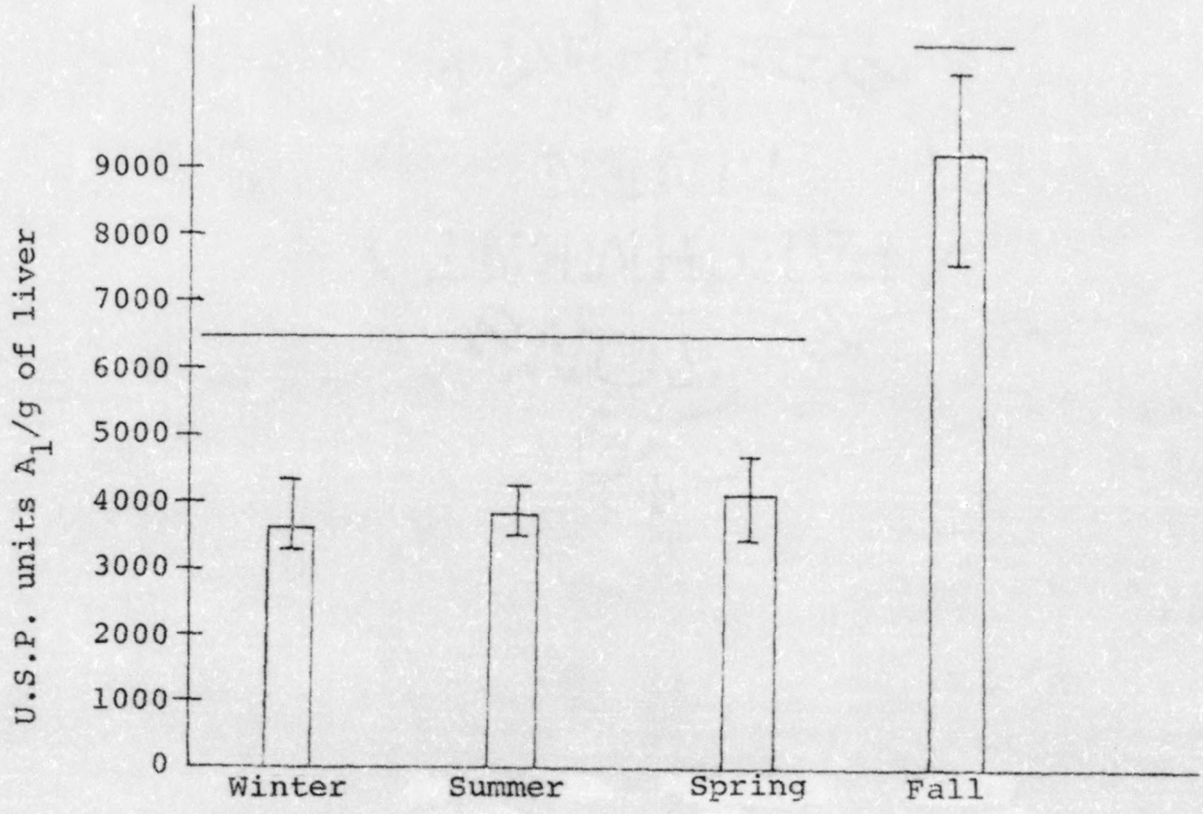
No. of Fish in Group	Type of Treatment	Mean Tot. Lgth. ± SEM[1] (cm)	Mean Tot. Wt. ± SEM (g)	Mean A_1 ± SEM[1] (U.S.P. units/ g liver)	Mean A_2 ± SEM[1] (U.S.P. units/ g liver)	
2	Initials+75	18±0	118±3	37±26	40±29	D[2]
5	Deficients	21±1	199±8	207±118	400±146	D
5	Luteins	21±1	176±8	2756±424	2716±518	E
2	Initials	19±0	122±4	3889±56	3762±34	E
5	Controls	21±0	179±6	4896±98	4543±338	E
5	Beta-carotenes	21±0	193±1	4921±997	3428±908	E

[1]SEM = Standard Error of the Mean

[2]Means with a common letter are not significantly different ($P<0.05$)

Figure 5. Mean vitamin A_1 levels (U.S.P. units/g of liver) of fish for the seasons of 1978-1979. Any means not spanned by the same line are significantly different ($P<0.05$)

Figure 6. Mean vitamin A_2 levels (U.S.P. units/g of liver) of fish for the seasons of 1978-1979. Any means not spanned by the same line are significantly different ($P<0.05$).


U.S.P. units A_1/g of liver
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Winter
Summer
Spring
Fall

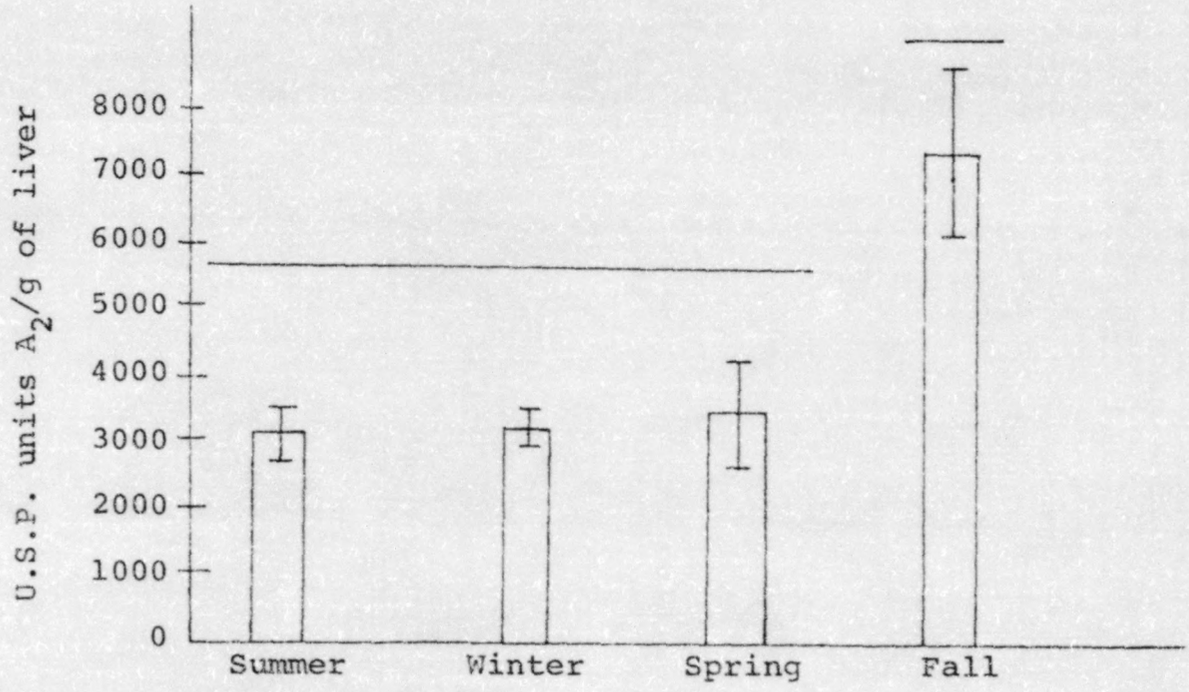
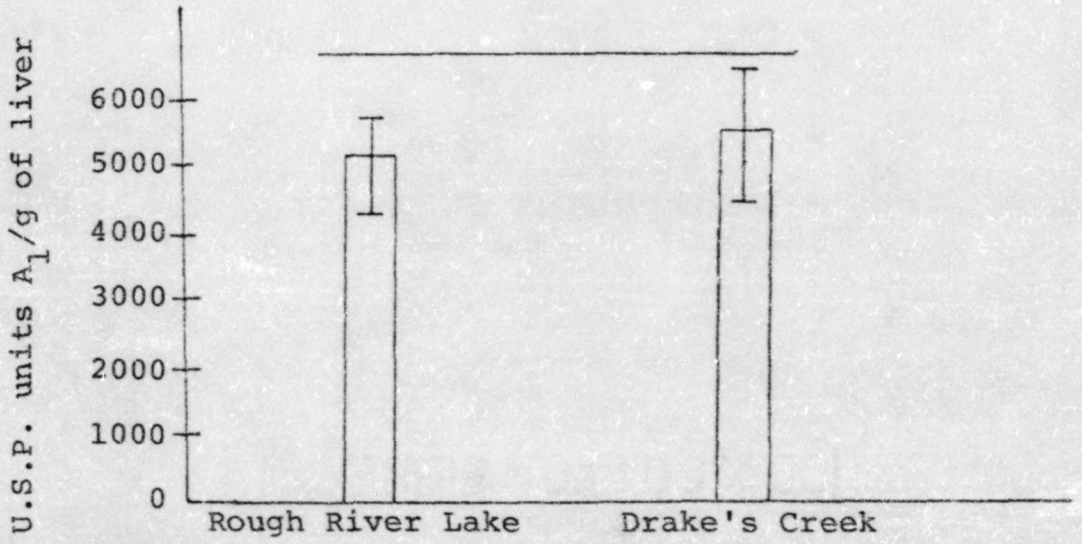

U.S.P. units A_2/g of liver
8000
7000
6000
5000
4000
3000
2000
1000
0
Summer
Winter
Spring
Fall

Figure 7. Mean vitamin A_1 levels (U.S.P. units/g of liver) of fish from Rough River Lake and Drake's Creek. Any means not spanned by the same line are significantly different ($P<0.05$).

Figure 8. Mean vitamin A_2 levels (U.S.P. units/g of liver) of fish from Rough River Lake and Drake's Creek. Any means not spanned by the same line are significantly different ($P<0.05$).


U.S.P. units A_1/g of liver
6000
5000
4000
3000
2000
1000
0
Rough River Lake
Drake's Creek

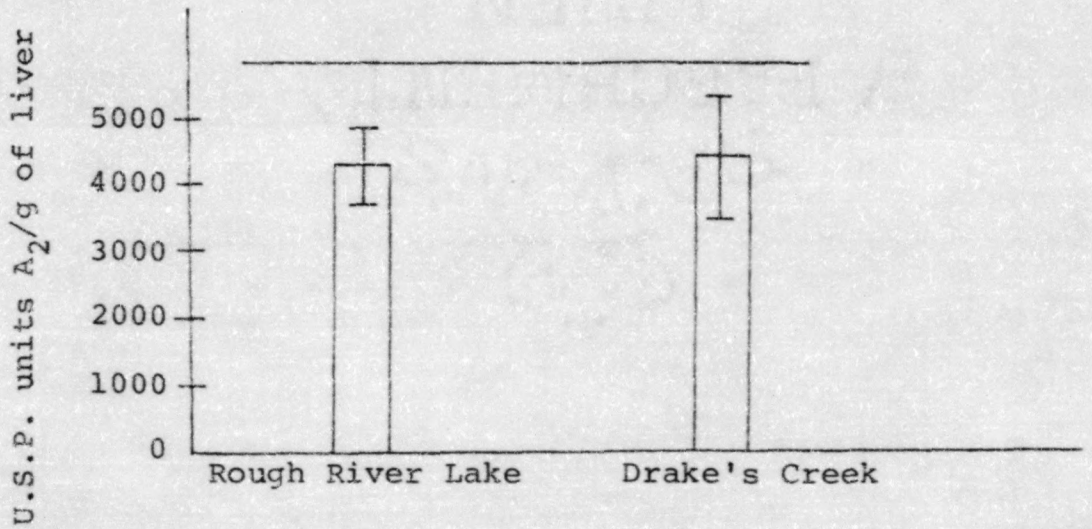

U.S.P. units A_2/g of liver
5000
4000
3000
2000
1000
0
Rough River Lake
Drake's Creek

Figure 9. Mean vitamin A_1 levels (U.S.P. units/g of liver) of goldfish by experimental treatment. Any treatments not spanned by the same line are significantly different ($P<0.05$).

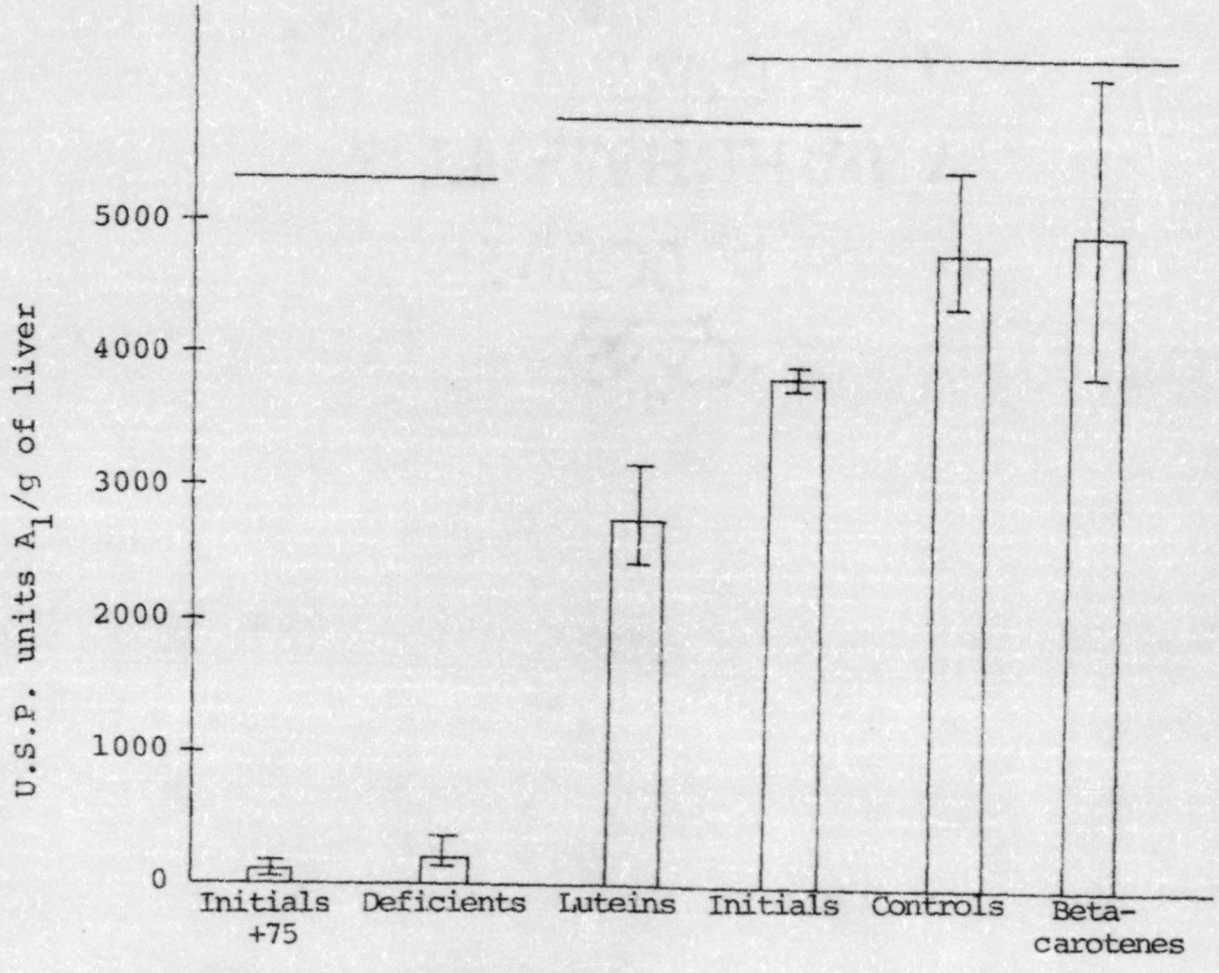
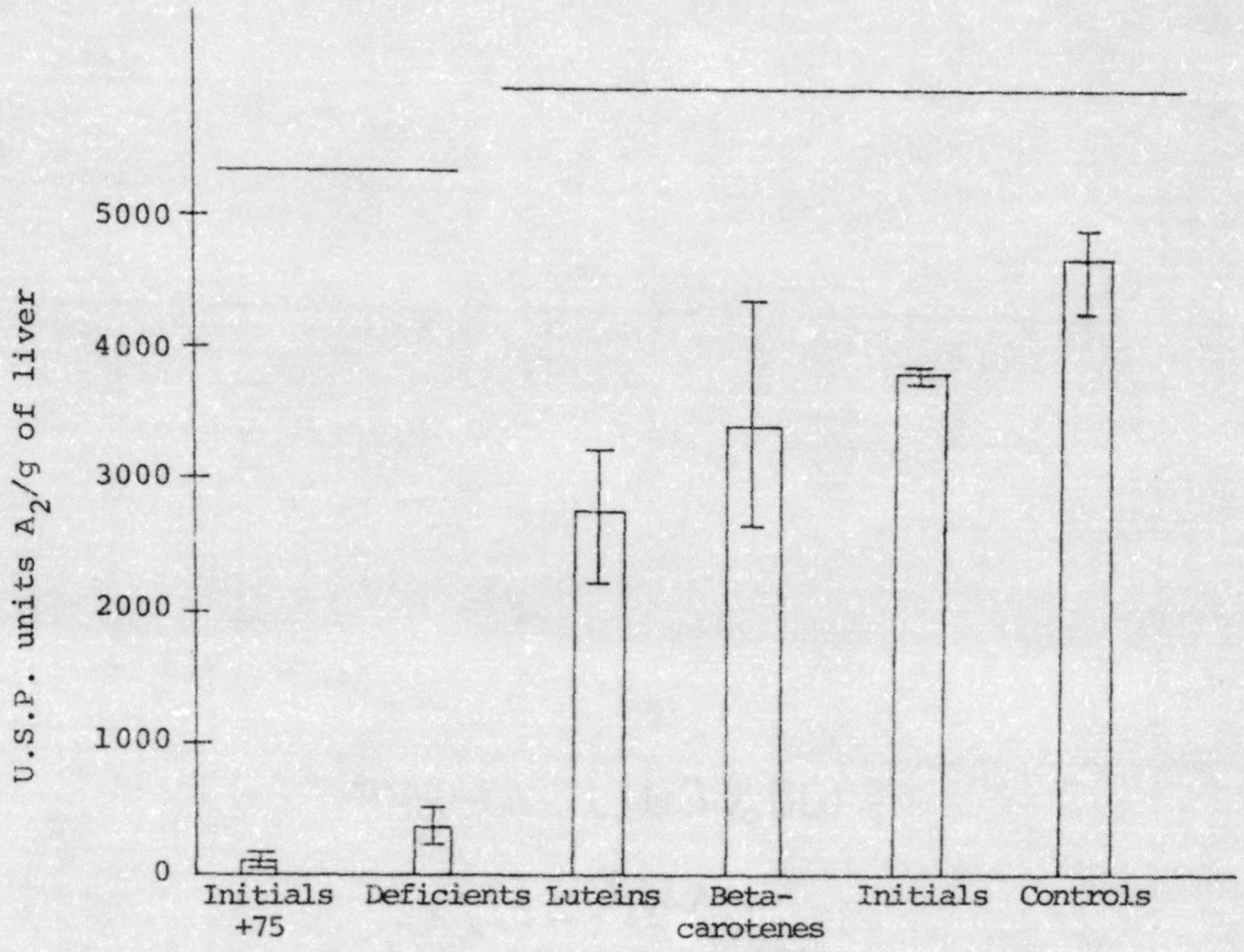

U.S.P. units A_1/g of liver
5000
4000
3000
2000
1000
0
Initials +75
Deficients
Luteins
Initials
Controls
Beta-carotenes

Figure 10. Mean vitamin A_2 levels (U.S.P. units/g of liver) of goldfish by experimental treatment. Any treatments not spanned by the same line are significantly different ($P<0.05$).


U.S.P. units A_2/g of liver
5000
4000
3000
2000
1000
0
Initials +75
Deficients
Luteins
Beta-carotenes
Initials
Controls

DISCUSSION

Vitamin A levels for the fish species observed in this study appeared to represent a physiological difference in vitamin synthesis between species rather than dietary or nutritional differences. Since all fish were exposed to and feeding upon similar food items, it was felt that diet was not the chief factor determining vitamin A levels. It also appeared that the northern hog sucker was more efficient in its ability to metabolize precursors to vitamin A and its subsequent storage than any other fish species in this study.

The occurrence of vitamin A_1 in higher concentration than vitamin A_2 in all species observed might possibly be due to a lag time in conversion of A_1 to A_2. Lovern et al. (1939) observed similar results with sturgeons, Acipenser sturio, and lampreys, Petromyzon fluviatilis. Morton and Creed (1939) reported A_2 levels to be slightly higher than A_1 in the perch, Perca fluviatilis, but found the opposite in both daces and chubs.

Since vitamin A is a fat-soluble vitamin, it was believed that the fish stored excess fat and vitamin A prior to the winter months. This, along with a readily available food supply, would suggest high vitamin A levels in the fall. The very low winter levels were believed to be due to the late sampling date, which may have occurred during the

1979 spawn. During this period, the fish would be catabolizing fat stores and subsequently reducing vitamin A levels. The slight increase in the spring probably resulted from increased feeding, whereas the slight summer drop was attributed to reduced feeding because of increasing water temperatures. Pugsley (1939), working with a halibut species, Hippoglossus hippoglossus, observed the same seasonal trends as reported here. However, since Pugsley's study was done with a marine species, only vitamin A_1 levels were observed and reported.

Since vitamin A_1 and A_2 levels from Drake's Creek and Rough River Lake fish were not significantly different, it was concluded that neither lotic nor lentic environments provide their inhabitants with a suitable advantage for vitamin A metabolism.

The data obtained in the laboratory study with goldfish indicates that vitamin A was synthesized from two precursors, beta-carotene and lutein. The conversion of beta-carotene into vitamin A, specifically A_2 in freshwater fish, was first identified by Morton and Creed (1939). Neilands (1947) also reported the conversion of beta-carotene to vitamin A in two marine species. Poston (1968) demonstrated that brook trout, Salvelinus fontinalis, were unable to convert beta-carotene to vitamin A, indicating perhaps a species specific metabolic pathway in the synthesis of vitamin A.

The observation of beta-carotene being converted to vitamin A_1 at a significantly greater rate than lutein would be expected on the basis of their molecular similarity. Likewise, the ring structure of lutein would provide only one-half the potential for its synthesis into vitamin A_1. This suggestion was supported by Gross and Budowski's (1966) report that lutein was devoid of provitamin A properties in two freshwater fish. Czeczuga and Czerpak (1976) reported beta-carotene and lutein conversion to vitamin A_1 but not A_2 in Carassius carassius and Leucaspius delineatus.

The statistically lower rate of conversion of lutein into vitamin A_1 and its similar rate of incorporation as beta-carotene into vitamin A_2 implied the possibility of lutein being synthesized directly into both vitamin A_1 and A_2. Baura, et al. (1973) and Baura and Das (1975) reported conversion of lutein to vitamin A_2 in Saccobranchus fossilis, an Indian freshwater species, but no direct conversion to vitamin A_1.

These precursors, beta-carotene and lutein, are found primarily in plant material as well as in crustaceans. Hagar and Stansky (1970) reported abundance of both beta-carotene and lutein in green algae. Gross and Budowski (1966), Grangaud et al. (1956), and Grangaud et al. (1957) reported another plant pigment, astaxanthin, as a precursor to vitamin A_1 and A_2 in Lebistes reticulatus and Gambusia holbrooki. This pigment was not used in the present study.

Lane (1950) reported a noncarotene provitamin A source from zooplankton oil that was converted to vitamin A by Limanda ferruginea.

From the data presented herein, it also appeared that vitamin A_1 may have been converted to A_2 since vitamin A_2 was found in those fish fed the control diet, containing vitamin A_1 only. This would, if true, agree with the work of Morton et al. (1947) who reported conversion of vitamin A_1 to A_2 in ling cod. Naito and Wilt (1962) also reported vitamin A_1 conversion to A_2 in sunfish (Lepomis spp.), as did Hata et al. (1973) for goldfish.

LITERATURE CITED

Ames, S.R., H.A. Risley and P.L. Harris. 1954. Simplified procedure for extraction and determination of vitamin A in liver. Anal. Chem. 26:1378-1381.

Aoe, H., I. Masuda, T. Mimura, T. Saito and A. Komo. 1968. Requirement of young carp for vitamin A. Bull. Jpn. Soc. Sci. Fish. 34:959-664.

Barua, A.B., H.T. Singh and R.C. Das. 1973. Conversion of lutein into dehydroretinol by the freshwater fish, Saccobranchus fossilis. Br. J. Nutr. 30:1-12.

Barua, A.B. and R.C. Das. 1975. Occurrence and conversion of anhydrolutein into dehydroretinol in a freshwater fish. Br. J. Nutr. 33:319-327.

Czeczuga, B. and R. Czerpak. 1976. Carotenoids in fish. VII. The kind of food and the content of carotenoids and vitamin A in Carassius carassius (L.) and Leucaspius delineatus (Heck). Acta. Hydrobiol. 18:1-21.

Dupree, H.K. 1966. Vitamins essential for the growth of channel catfish, Ictalurus punctatus. Bur. Sport Fish. Wild. Tech. Pap. 7:1-12.

Granguad, R., P. Vignais, R. Massonet and J.I. Moatti. 1956. The biosynthesis of vitamin A by Gambusia holbrooki. C. R. Acad. Bulg. Sci. 243:1170-1172.

_____, _____, _____ and _____. 1957. Biogenesis of vitamin A in fish. II. Neoformation of vitamin A_1 (retinol) and A_2 (dehydroretinol) in Gambusia holbrooki. Bull. Soc. Chim. Biol. 39:1271-1278.

Gross, J. and P. Budowski. 1966. Conversion of carotenoids into vitamin A_1 and A_2 in two species of freshwater fish. Biochem. J. 101:747-753.

Hagar, A. and H. Stransky. 1970. The carotenoid pattern and the occurrence of the light-induced xanthophyll cycle in various classes of algae. III. Green algae. Arch. Microbiol. 72:68-83.

Halver, J.E. 1957. Nutrition of salmonid fishes. III. Water-soluble vitamin requirements of chinook salmon. J. Nutr. 62:225-243.

_____. 1972. Fish Nutrition. Academic Press, New York. 713 p.

Hata, M., M. Hata and T. Onishi. 1973. Conversion of beta-carotene and retinol$_1$ to retinol$_2$ in freshwater fish. Tohoku J. Agric. Res. 24:197-204.

Hopkins, F.G. 1912. Feeding experiments illustrating the importance of accessory factors in normal diets. J. Physiol. 44:425-460.

Horwitz, W. 1975. Official Methods of Analysis of the Association of Official Analytical Chemists. 12th Ed. Association of Analytical Chemists, Washington, D.C. 1272 p.

Jones, J.H., E.B. Bullard and A. Rodriguez. 1966. Vitamin A in the nutrition of goldfish, Carassius auratus. Fed. Proc. 25:546.

Lane, C.E. 1950. A noncarotene provitamin A for fishes. Science. 111:471-472.

Lovern, J.A., R.A. Morton and J. Ireland. 1939. The distribution of vitamins A_1 and A_2. II. Biochem. J. 33:325-329.

Morton, R.A. and R.H. Creed. 1939. The conversion of carotene to vitamin A_2 by some freshwater fishes. Biochem. J. 33:318-324.

Morton, R.A., M.K. Salah and A.L. Stubbs. 1947. Retinene$_2$ and vitamin A_2. Nature 159:744.

Naito, K. and F.H. Wilt. 1962. The conversion of vitamin A_1 to retinene$_2$ in a freshwater fish. J. Biol. Chem. 237:3060-3064.

Neilands, J.B. 1947. The conversion of carotene to vitamin A in the fish. Arch. Biochem. Biophys. 13:415-419.

Osborne, T.B. and L.B. Mendel. 1914. The influence of cod liver oil and some other fats on growth. J. Biol. Chem. 17:401-408.

Pennak, R.W. 1978. Freshwater Invertebrates of the United States. 2nd Ed. John Wiley & Sons, New York. 803 p.

Poston, H.A. 1968. The conversion of beta-carotene to vitamin A by fingerling brook trout. Fish. Res. Bull. 32:42-43.

Pugsley, L.I. 1939. Vitamin A and D potencies of liver and intestinal oils of halibut, Hippoglossus hippoglossus. J. Fish. Res. Board Can. 4:396-404.

Smith, G.M. 1950. The Freshwater Algae of the United States. 2nd Ed. McGraw-Hill Book Company, New York. 719 p.

United States Pharmacopeial Convention. 1955. Pharmacopeia of the United States of America. XV Revision. Mark Publishing Company, Pennsylvania. 1178 p.

West, E.S., W.R. Todd, H.J. Mason and J.T. Van Bruggen. 1966. Textbook of Biochemistry. 4th Ed. The Macmillan Company, New York. 1595 p.

Yates, F. 1933. The analysis of replicated experiments when the field results are incomplete. Emp. J. Exp. Agric. 1:129-142.