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

Benjamin Del Tito Jr.
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
Presented to
the Faculty of the Department of Biology
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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A COMPARISON OF VITAMIN A₁ AND A₂ LEVELS
AND THE ROLE OF BETA-CAROTENE AND LUTEIN
IN THE SYNTHESIS OF VITAMIN A IN FRESHWATER FISHES

Recommended April 17, 1980
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Robert D. Hoyt
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Approved April 16, 1980
(Date)

Elmer Gray
Dean of the Graduate College
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I would like to express my sincere appreciation to the members of my committee, Drs. Frank R. Toman and Larry N. Gleason, for their guidance throughout the course of this study. I would especially like to thank my committee chairman, Dr. Robert D. Hoyt, who provided a constant source of constructive criticism and guidance during every stage of this research.

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Special thanks go to Mr. Charles Bowers and Mr. Dan Brewer of the Kentucky Department of Fish and Wildlife Resources for the goldfish used in the study. Dr. Larry N. Gleason's assistance in field collections along with fellow students Yui Tan Chung, Greg Kindschi, Bill Kruskamp, Allen Robison and Dennis Webb are appreciated. Dr. Frank R. Toman's assistance in the laboratory analysis is appreciated.

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The facilities and space provided by the Department of Biology and Western Kentucky University are acknowledged.

Finally, very special thanks are due to my wife, who provided endless encouragement and understanding.
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A COMPARISON OF VITAMIN A₁ AND A₂ LEVELS AND THE ROLE OF BETA-CAROTENE AND LUTEIN IN THE SYNTHESIS OF VITAMIN A IN FRESHWATER FISHES

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 A₁ and A₂ 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 A₁ and A₂ by their ability to metabolize vitamin A from their environment. Seasonally, vitamin A₁ and A₂ 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 xerophthalmia 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, xerophthalmia, 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 A₂, in selected freshwater species. Morton et al. (1947), Naito and Wilt (1962), and Hata et al. (1973) reported conversion of vitamin A₁ to A₂ in three freshwater species.

The objectives of this study were to determine naturally occurring concentrations of vitamin A₁ and A₂ 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 m³ 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.
Figure 2. Map of Rough River Lake, Kentucky, showing the location of the collecting station.
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 fluorescent 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 commer-
cial grade, high protein trout chow containing 350 U.S.P.
units of vitamin A/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-carotenates) 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-caroten was purchased
from and supplemented into the diet by ICN. Lutein was
extracted from egg yolk and provided to ICN for supplemen-
tation. 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 A₁ 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 A₁ 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 A₂ 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 A₁ and A₂ in two stream fishes (Drake's Creek) and three lake fishes (Rough River Lake) for 1978-1979.

<table>
<thead>
<tr>
<th>Collection Location</th>
<th>Species</th>
<th>Mean Tot. Lgth. ± SEM (cm)</th>
<th>Mean Tot. Wt. ± SEM (g)</th>
<th>Mean A₁ ± SEM (U.S.P. units/g liver)</th>
<th>Mean A₂ ± SEM (U.S.P. units/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drake's Creek</td>
<td>Hog Sucker</td>
<td>28 ± 1</td>
<td>208 ± 24</td>
<td>12507 ± 4402</td>
<td>11141 ± 4295</td>
</tr>
<tr>
<td></td>
<td>Golden Redhorse</td>
<td>30 ± 3</td>
<td>329 ± 100</td>
<td>5374 ± 1513</td>
<td>3067 ± 1367</td>
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<tr>
<td>Fall</td>
<td>Rough River Lake</td>
<td>Black Buffalo Smth. Buffalo Flathead Catfish</td>
<td>70 ± 6</td>
<td>52 ± 1</td>
<td>71 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>Drake's Creek</td>
<td>Hog Sucker Golden Redhorse</td>
<td>28 ± 0</td>
<td>31 ± 4</td>
<td>2866 ± 27</td>
</tr>
<tr>
<td>Collection</td>
<td>Location</td>
<td>Species</td>
<td>Mean Lgth. ± SEM (cm)</td>
<td>Mean Wt. ± SEM (g)</td>
<td>Mean $A_1$ ± SEM (U.S.P. units/g liver)</td>
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<tr>
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<td>-----------</td>
<td>-------------</td>
<td>-----------------------</td>
<td>--------------------</td>
<td>----------------------------------------</td>
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<tr>
<td>Winter</td>
<td>Lake</td>
<td>Black Buffalo</td>
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<td>6016 ± 9</td>
<td>3249 ± 1072</td>
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<td></td>
<td></td>
<td>Smmth. Buffalo</td>
<td>53 ± 2</td>
<td>2582 ± 6</td>
<td>2599 ± 448</td>
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<td></td>
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<td>Flathead Catfish</td>
<td>72 ± 4</td>
<td>4965 ± 9</td>
<td>4101 ± 1004</td>
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<td>Drake's</td>
<td>Creek</td>
<td>Hog Sucker</td>
<td>24 ± 2</td>
<td>154 ± 32</td>
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<td>Golden Redhorse</td>
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<td>280 ± 6</td>
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<td>Black Buffalo</td>
<td>83 ± 2</td>
<td>8981 ± 6</td>
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<td>51 ± 1</td>
<td>2206 ± 7</td>
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<td>Flathead Catfish</td>
<td>36 ± 1</td>
<td>467 ± 56</td>
<td>4657 ± 1148</td>
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<td>Summer</td>
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<td>Hog Sucker</td>
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<td>Golden Redhorse</td>
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<td>196 ± 23</td>
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Table 1. Continued.

<table>
<thead>
<tr>
<th>Collection Location</th>
<th>Species</th>
<th>Mean Lgth. ± SEM (cm)</th>
<th>Mean Wt. ± SEM (g)</th>
<th>Mean A₁ ± SEM (U.S. P. units/g liver)</th>
<th>Mean A₂ ± SEM (U.S. P. units/g liver)</th>
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<tr>
<td>Summer Lake</td>
<td>Rough Black River</td>
<td>Buffalo</td>
<td>76±6</td>
<td>6872±10</td>
<td>4783±1050</td>
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<tr>
<td></td>
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<td>Smmth.</td>
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<td>1901±7</td>
<td>2562±454</td>
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<td></td>
<td>Flathead</td>
<td>43±4</td>
<td>965±26</td>
<td>4458±321</td>
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<tr>
<td></td>
<td></td>
<td>Catfish</td>
<td>43±4</td>
<td>965±26</td>
<td>4458±321</td>
</tr>
<tr>
<td></td>
<td>Drake's Hog Creek</td>
<td>Hog</td>
<td>28±1</td>
<td>222±13</td>
<td>7431±865</td>
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<tr>
<td></td>
<td></td>
<td>Sucker</td>
<td>28±1</td>
<td>222±13</td>
<td>7431±865</td>
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<tr>
<td></td>
<td></td>
<td>Golden</td>
<td>30±0</td>
<td>292±18</td>
<td>3386±343</td>
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<tr>
<td></td>
<td></td>
<td>Redhorse</td>
<td>30±0</td>
<td>292±18</td>
<td>3386±343</td>
</tr>
</tbody>
</table>

| Grand Species Means| Rough Black River| Buffalo               | 76±1               | 6872±10                             | 6142±1184                           |
|                     | Smmth.            | Buffalo               | 52±0               | 2268±6                              | 3546±433                            |
|                     |                   | Flathead              | 56±5               | 2628±8                              | 5350±476                            |

*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 ($P<0.05$).
Seasonally, vitamin A₁ 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 A₂ 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 A₁ (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 A₂ 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 A₁ or A₂ levels.

**Laboratory Data**

In goldfish the Beta-carotenes had the highest average vitamin A₁ 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 A₂ 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 A₂ 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.

<table>
<thead>
<tr>
<th>No. of Fish in Group</th>
<th>Type of Treatment</th>
<th>Mean Tot. Lgth. ± SEM(^1) (cm)</th>
<th>Mean Tot. Wt. ± SEM (g)</th>
<th>Mean $A_1$ ± SEM (^1) (U.S.P. units/g liver)</th>
<th>Mean $A_2$ ± SEM (^1) (U.S.P. units/g liver)</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td>Initials+75</td>
<td>18±0</td>
<td>118±3</td>
<td>37±26</td>
<td>40±29</td>
</tr>
<tr>
<td>5</td>
<td>Deficients</td>
<td>21±1</td>
<td>199±8</td>
<td>207±118</td>
<td>400±146</td>
</tr>
<tr>
<td>5</td>
<td>Luteins</td>
<td>21±1</td>
<td>176±8</td>
<td>2756±424</td>
<td>2716±518</td>
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<td>2</td>
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<td>122±4</td>
<td>3889±56</td>
<td>3762±34</td>
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<td>Controls</td>
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<td>4896±98</td>
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<td>21±0</td>
<td>193±1</td>
<td>4921±997</td>
<td>3428±908</td>
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</table>

\(^1\) 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 ($P<0.05$).
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 ($P<0.05$).
Figure 9. Mean vitamin A₁ 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).
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 $\mu g$ of liver

<table>
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<td>Luteins</td>
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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 A₁ levels were observed and reported.

Since vitamin A₁ and A₂ 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 A₂ 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 A₁ may have been converted to A₂ since vitamin A₂ was found in those fish fed the control diet, containing vitamin A₁ only. This would, if true, agree with the work of Morton et al. (1947) who reported conversion of vitamin A₁ to A₂ in ling cod. Naito and Wilt (1962) also reported vitamin A₁ conversion to A₂ in sunfish (*Lepomis* spp.), as did Hata et al. (1973) for goldfish.
LITERATURE CITED


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