

## Nutritional Implications of Tryptophan Catabolizing Enzymes in Several Species of Trout and Salmon (40795)

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Salmonids require a dietary source of both niacin and tryptophan for growth, survival, and prevention of dermal lesions. According to Halver (4), the level of niacin required for optimum growth and maximum liver storage in salmon (150–200 mg/kg of feed) is twice that required in trout. The salmonid requirement for tryptophan (0.5% of dietary protein) is only half that required by the rat (4). Halver (4) suggested that the species difference in dietary requirement for niacin by fishes may be explained by a difference in rate of biosynthesis of nicotinamide adenine dinucleotide (NAD) from the dietary precursor tryptophan.

The efficiency of the conversion of tryptophan to NAD varies widely among species of vertebrates (5, 6) and between genetically selected low and high niacin-requiring strains of the chick (7), and is inversely related to the activity of liver picolinic acid carboxylase (PC). This enzyme (PC) decarboxylates 2-amino-3-carboxymuconic-6-semialdehyde, the unstable intermediate oxidation product formed by the action of 3-hydroxyanthranilic acid oxygenase (3HAA) on 3-hydroxyanthranilic acid, and diverts it toward formation of CO<sub>2</sub> (by the way of the glutaryl coenzyme A pathway) rather than toward quinolinic acid, the immediate precursor of nicotinic acid.

Henderson and Swan (8) proposed the use of the ratio of liver 3HAA:PC to predict the effectiveness of dietary tryptophan as a replacement for dietary niacin; a high ratio would indicate efficient replacement.

Poston and DiLorenzo (9) reported that the brook trout (*Salvelinus fontinalis*) had a high level of liver PC activity and a narrow 3HAA:PC ratio (1.49:1). Growth data from a nutritional study (9) supported the liver enzyme evidence that dietary tryptophan is

not an efficient precursor of niacin for the brook trout.

A knowledge of the liver 3HAA:PC ratio of various salmonids should be useful in predicting possible species and generic variations in requirements for dietary niacin. The present study was designed to compare the ratio of the activity of liver PC and 3HAA in a total of five species in three genera of the Salmonidae.

*Materials and methods. Experimental fish.* Livers for measurement of 3HAA and PC activities were taken from 16 adult fish of each of the following species: brook trout (*Salvelinus fontinalis*), mean body weight, 355 g; lake trout (*Salvelinus namaycush*), 530 g; rainbow trout (*Salmo gairdneri*), 400 g; Atlantic salmon (*Salmon salar*), 160 g; and coho salmon (*Oncorhynchus kisutch*), 25 g. Except for the coho salmon, these fish (predominantly males) had been reared at the Tunison Laboratory of Fish Nutrition, and all had been fed a commercially prepared production diet which contained 0.9% of total protein as tryptophan, and a total of 420 mg niacin/kg of feed. The coho salmon had been reared at the U.S. Fish and Wildlife Salmon Cultural Development Center, Longview, Washington.

*Fish liver enzyme assays.* Enzyme assays were conducted according to the method of Mehler *et al.* (10). We made determinations on four pooled samples for each *Salvelinus* and *Salmo* species, using 0.5 g liver from each of four individual fish per sample. The livers from each composite sample were cooled on ice and homogenized immediately in a glass and Teflon homogenizer with 0.9% NaCl to make a 20% homogenate. The homogenates were stored at 4°C overnight before centrifugation at 15,000g and 4°C for 15 min. The resulting supernatants were assayed for 3HAA and PC at

25°C and pH 7.5. A recording spectrophotometer was used to measure change in absorbance at 360 nm.

Sufficient livers from coho salmon to make four 2-g samples were quick-frozen with dry ice in Longview, Washington, and kept frozen until homogenized at the Tunison Laboratory.

The reaction mixture contained 0.20 ml of 10% supernatant; 0.50 ml of 1 M phosphate buffer (pH 7.5); 0.10 ml of 0.01% 3-hydroxyanthranilic acid (in  $10^{-3}$  N  $H_2SO_4$ ), and 2.20 ml of glass-distilled water. The rate of increase in absorbance after the addition of the substrate (3-hydroxyanthranilic acid), was measured and converted to micromoles of 2-amino-3-carboxymuconic-6-semialdehyde/gram of wet liver/hour, by using the molar extinction coefficient of 45 (11). After maximum absorbance was reached, the ensuing decrease in absorbance, which was caused by disappearance of the semialdehyde, was converted to micromoles/gram wet liver/hour to represent liver PC activity. Statistical significance of intergeneric and interspecific differences in the ratio of fish liver 3HAA to PC activity (3HAA:PC) was established by analysis of variance of Duncan's multiple range test (12).

**Results.** Ratios of picolinic acid carboxylase to 3-hydroxyanthranilic acid oxygenase (Table I.). The activities of 3HAA and PC were each proportional to the amount of 10% supernatant used. Differences in the liver enzyme ratios (3HAA:PC) among the genera *Salvelinus* (lake trout and brook trout), *Salmo* (rainbow trout and Atlantic salmon) and *Oncorhynchus* (coho salmon) were significant ( $P < 0.01$ ), but there were no within-genus differences ( $P > 0.05$ ). The 3HAA:PC ratio was widest in *Oncorhynchus* and narrowest in *Salmo*.

**Discussion.** Results of this study support and expand the findings of previous work (9) in showing that the brook trout has a narrow liver 3HAA:PC ratio. The present data, however, show that brook trout had considerably lower enzyme activities of both the 3HAA and PC enzymes than were reported earlier (9). In the present study, the 3HAA:PC ratio was somewhat higher

TABLE I. COMPARATIVE ACTIVITY OF 3-HYDROXYANTHRANILIC ACID OXYGENASE (3HAA) AND PICOLINIC CARBOXYLASE (PC) IN LIVERS OF VARIOUS SALMONID FISHES

Genus	3HAA <sup>a</sup>	PC <sup>b</sup>	3HAA:PC <sup>c</sup>
<i>Salvelinus</i>			
Lake trout	76	27.4	2.77 <sup>d</sup>
Brook trout	35	15.1	2.27 <sup>d</sup>
<i>Salmo</i>			
Atlantic salmon	35	29.2	1.20 <sup>e</sup>
Rainbow trout	25	17.0	1.46 <sup>e</sup>
<i>Oncorhynchus</i>			
Coho salmon	51	14.9	3.42 <sup>f</sup>
Pooled SE	6.42	1.47	0.21

<sup>a</sup> Appearance ( $\mu$ mol of 2-amino-3-carboxymuconic-6-semialdehyde/g wet liver/hr).

<sup>b</sup> Disappearance ( $\mu$ mol of semialdehyde/g wet liver/hr).

<sup>c</sup> Ratios not sharing a common superscript letter differ significantly ( $P < 0.05$ ). Each value is the mean of four samples with four livers/sample.

than reported previously. These differences possibly were due to the longer interim (overnight) between the preparation of liver homogenates and the completion of the enzyme assays in the present investigation; or to differences in size, age, sex, and sexual maturation of the experimental fish; or to composition of the diet. Also, the use of a larger number of fish in the present study may have resulted in more precision than that in the previous study (9).

In general, there was an inverse relationship between the liver 3HAA:PC ratio and the published requirement for dietary niacin of several vertebrate animals (Table II). Further studies are needed to explore this relationship more fully. This comparison suggests, however, that all species of salmonid fishes examined in the present study are considerably less efficient in converting tryptophan to niacin than is the cat. Since most workers believe that the cat must obtain all of its niacin intact from the diet (6), salmonid fishes most likely must do the same. It is not known if the differences in the liver enzyme ratios (3HAA:PC) in these species of fish account for any measureable difference in the quantitative dietary niacin requirement. However, if fish must obtain all their niacin from the diet and, therefore, synthesize essentially no niacin from tryptophan,

TABLE II. COMPARISON OF LIVER ENZYME RATIOS, DIETARY NIACIN NEEDS, AND POTENTIAL NIACIN-SPARING EFFECT OF TRYPTOPHAN IN VARIOUS SPECIES

Species	3HAA:PC	Niacin need (mg/kg feed)	Relative niacin-sparing action of tryptophan <sup>a</sup>
Rat	273 <sup>b</sup>	0	—
Chick, low niacin	48 <sup>c</sup>	5 <sup>c</sup>	9.6
Chick, high niacin	27 <sup>c</sup>	15 <sup>c</sup>	1.9
Duck	5.3 <sup>c</sup>	40 <sup>c</sup>	0.13
Cat	5 <sup>b</sup>	45 <sup>d</sup>	0.11
Brook trout and lake trout	2.5	88 <sup>e</sup>	0.03
Turkey	1.6 <sup>c</sup>	70	0.02
Rainbow trout and Atlantic salmon	1.3	88 <sup>e</sup>	0.015
Coho salmon	3.4	175 <sup>e</sup>	0.02

<sup>a</sup> Obtained by dividing 3HAA:PC by dietary niacin need for each species.

<sup>b</sup> Taken from Ikeda *et al.* (5).

<sup>c</sup> Taken from DiLorenzo (7).

<sup>d</sup> NRC. Nutrient requirements of cats (6).

<sup>e</sup> Mean of published niacin needs (4).

tophan, these ratios apparently do not predict differences in the dietary requirements for niacin among the species studied.

*Summary and conclusions.* Data obtained from assays from activity of 3HAA and PC in livers of five species of trout and salmon indicate that, in general, dietary tryptophan is not an effective precursor of niacin, for which a dietary requirement exists. Intergeneric differences in this relationship may or may not suggest differences in the quantitative requirements for dietary niacin by these individual species of salmonids.

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Received September 20, 1979. P.S.E.B.M. 1980, vol. 163.