Tryptophan Conversion to Niacin in the Brook Trout (*Salvelinus fontinalis*) (37537)

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(Introduced by M. L. Scott)

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Salmonids require dietary niacin for growth and survival (1, 2), prevention of dermal abnormalities (3), and control of fat deposition (4).

According to Halver (5), niacin requirements for optimum growth response and maximum liver storage in salmon are apparently twice those of trout. He postulated that this generic difference may be caused by species metabolic differences and that the comparatively long time (10–14 wk) required to produce a niacin deficiency syndrome in several fish species fed a niacin-deficient diet may be explained by a conversion of tryptophan to niacin as many animals biosynthesize significant niacin from this source.

The efficiency of this conversion varies widely among species (6, 7) and between genetically selected strains of the chicken (8). This variation apparently is caused by inherent differences in liver levels of picolinic acid carboxylase (PC), the enzyme which diverts 2-amino-3-carboxymuconic-6-semialdehyde, one of the tryptophan-nicotinic acid adenine dinucleotide (NAD) intermediates, toward CO_2 via the glutaryl-CoA pathway rather than toward quinolinic acid, the immediate precursor of nicotinic acid.

The enzyme, 3-hydroxyanthranilic acid oxygenase (3HAA; EC 1.13.1.6) catalyzes the formation of 2-amino-3-carboxymuconic-6-semialdehyde, the unstable intermediate common to the glutaryl-CoA and NAD pathways, from 3-hydroxyanthranilic acid.

Henderson and Swan (9) suggested the use of the ratio of liver 3HAA/PC activity to measure the tryptophan-to-NAD conversion efficiency, whereby a high ratio would signify an efficient conversion. DiLorenzo (8) found a high correlation between this measurement and growth in chickens fed niacin-deficient diets.

A knowledge of the ratio of trout liver 3HAA/PC activities should be useful in estimating the effectiveness of tryptophan as a dietary source of niacin for salmonids. The present study was designed to (a) examine the effect of supplementing either niacin or tryptophan in a basal, semipurified diet low in niacin (3 μg/g) on the growth, survival and lipid composition of juvenile brook trout (*Salvelinus fontinalis*) and (b) determine the level of liver PC and 3HAA.

Materials and Methods. Nutritional study. Brook trout, initially 1.2 g average weight, were fed either (a) the basal diet, a modification of that described by Wolf (10) which contained approximately 0.2% tryptophan in 16.4% "vitamin-free" casein (wet basis) and 3 μg of niacin/g, (b) the basal diet plus 240 μg supplemental niacin/g of diet, or (c) the basal diet plus 1.5 g supplemental L-tryptophan/100 g of diet.

Each diet was fed to duplicate tanks of 500 fish each in 8.3° well water for 20 wk. The fish were weighed biweekly and were fed to satiation 3 times daily.

At 20 wk, samples of fish were analyzed for carcass ash, fat, protein and moisture and for liver lipid.

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Fish liver enzyme assays. Livers from 9 adult brook trout, 600 g average weight each, were used to measure 3HAA and PC activities.

Enzyme assays were conducted using the spectrophotometric method of Mehler, McDaniel and Hundley (11). Determinations were performed on pooled livers using 3 fish/sample. Immediately after removal, the livers were cooled in ice and homogenized with 0.9% NaCl to make a 20% homogenate in a glass and Teflon homogenizer. The homogenates were centrifuged at 20,000*g* and 2° for 20 min. The resulting supernatants were held in an ice bath until assayed for 3HAA and PC, at 25°. A Guilford recording spectrophotometer was used to measure change in optical density per minute.

The reaction mixture contained: 0.20 ml of 10% homogenate; 0.50 ml of 0.1 *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 optical density, after the addition of the final ingredient (3-hydroxyanthranilic acid) was measured and converted to micromoles of 2-amino-3-carboxymuconic-6-semialdehyde per hour per gram of wet liver using the extinction coefficient of 45,000 (12). The ensuing decrease in optical density per min was due to disappearance of the semialdehyde and was converted to micromoles per hour per gram of wet liver and represented liver PC activity.

Results. Nutritional study. Body size. The dietary niacin deficiency caused a significant body weight reduction ($p < 0.01$; Table I). This growth depression, evident by 8 wk, intensified progressively during the last 12 wk of the experiment. The supplemental tryptophan-fed fish were smaller than the basal group early in the study, but gradually converged with, and then surpassed them in the final 4 wk.

Liver size (Table I). The larger hepatosomatic index of the supplemental tryptophan fish probably is primarily a reflection of more liver tissue/fish while the intermediate index of the basal group is probably a reflection of their smaller body size.

Mortality. The overall mean biweekly mortality was less than 0.5% and there were no significant differences among dietary groups.

Whole body and liver fat (Table I). Supplementing with either niacin or tryptophan suppressed whole carcass lipid while only tryptophan produced a lower liver lipid content.

Fish liver enzyme activity. Effect of homogenate level on enzyme activities. The activities of 3HAA and PC were each proportional to the amount of 10% homogenate used.

Picolinic acid carboxylase and 3-hydroxyanthranilic acid oxygenase (Table II). Though variable in amounts, each of the three pooled liver samples show a high level of PC in relation to 3HAA activity.

Discussion. The high liver PC activity and the narrow 3HAA/PC ratio (Table II) indicate that the brook trout is an inefficient converter of L-tryptophan to niacin. The intermediate semialdehyde is metabolized primarily via the glutaryl-CoA pathway. These findings corroborate the growth data of this study which show that dietary tryptophan is not an efficient niacin precursor for the brook trout. They do not support the postulate (5) that dietary tryptophan delays the appearance of a niacin deficiency syndrome in fish.

TABLE I. Effect of Supplemental Niacin and L-Tryptophan on Growth and Fat Content of Brook Trout.

Diet description	Final body wt (g)[a]	Liver wt (mg)	Body fat (%)	Liver fat (mg/g wet liver)
Basal	5.98 ± 0.05	118 ± 2.2	5.2 ± 0.15	80.6 ± 3.95
Basal + 240 ppm niacin	7.37 ± 0.27	110 ± 3.60	4.6 ± 0.15	83.4 ± 7.30
Basal + 1.5% L-tryptophan	6.21 ± 0.01	138 ± 0.0	4.0 ± 0.10	68.5 ± 1.80

[a] Mean ± standard error of mean.

TABLE II. Picolinic Acid Carboxylase (PC) and 3-Hydroxyanthranilic Acid Oxygenase (3HAA) Activity in Pooled Brook Trout Livers.

Liver samples	3HAA[a]	PC[b]	3HAA/PC
1	139	100	1.39
2	94	91	1.03
3	70	39	1.80
	101.0 ± 20[c]	76.7 ± 19	1.41 ± 0.22

[a] Appearance (μmoles of 2-amino-3-carboxymuconic-6-semialdehyde/g wet liver/hr).

[b] Disappearance (μmoles of semialdehyde/g wet liver/hr).

[c] Mean ± standard error of mean for activity. Three livers/sample.

The liver PC activity of the brook trout is similar to that of the cat (*Felis domestica*), which requires a dietary source for all its niacin (6) and has a liver PC activity of about 50.5 μmoles of substrate converted/g/wet liver/hr in contrast to 1.57 for the rat (*Ratus ratus*) (6) which can metabolize all its niacin from dietary tryptophan.

Interestingly, the turkey (*Meleagris gallopavo*) has a 3HAA/PC ratio of 1.6 (8) compared with 1.4 for the brook trout. The dietary niacin requirement for the turkey is 70 mg/kg of feed (8), which approaches the tentative requirement of 75–100 mg/kg of feed for trout (13).

Summary and conclusions. Data on growth, body composition and liver 3HAA and PC assays indicate that L-tryptophan is an inefficient precursor of dietary niacin for the brook trout.

The brook trout has a high liver PC activity. While this enzyme activity, alone, indicates a high requirement for dietary niacin, the 3HAA/PC ratio is a more meaningful indicator of the efficiency of the tryptophan-to-niacin conversion in the brook trout and other species.

An investigation of the PC and 3HAA activities of other salmonids should help elucidate interspecies and intergeneric variations in niacin requirements.

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