B Vitamin Requirements of Carp—III. Requirement for Biotin

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Requirements of salmonoid fishes for biotin have been investigated on rainbow trout^{1,2)}, brook trout¹⁾, brown trout¹⁾ and chinook salmon^{3,4)} and it has been demonstrated that the brown trout fed a biotin-deficient diet developed "blue slime" disease⁵⁾. Comparatively little is known, however, on the requirements of warm-water fishes for biotin. DUPREE⁶⁾ observed with channel catfish that there was no difference in weight gain or mortality between the two groups, biotin deficient and biotin supplemented. TOMIYAMA *et al.*⁷⁾ reported that the goldfish showed retarded growth and decrease of biotin content in the hepatopancreas by a deficiency of this vitamin in diet.

In the previous papers^{8,9)}, the requirements of young carp for pyridoxine, riboflavin and pantothenic acid were reported. The present study was carried out to see the role of biotin in carp nutrition. The young carp were reared with the diets containing grading amounts of biotin and were determined growth, mortality, vitamin content in the hepatopancreas and deficiency symptoms. Quantitative requirement of young carp for biotin determined tentatively by weight gain and by content in the hepatopancreas of the vitamin was 0.02–0.03 mg per kg of body weight per day.

Experimental

Basal diet and feeding method The basal diet devised by HALVER *et al.*¹⁰⁾ for estimation of vitamin requirements of trouts was used after a slight modification. It consisted of casein 50, soy-bean oil 3, cod liver oil 2, dextrin 15, α -starch 20, cellulose powder 5, mineral mixture 4 and vitamin mixture 1. Corn oil in Halver diet was substituted by soy-bean oil. The commercial vitamin free casein was boiled two times with ethanol to remove residual vitamins completely. The basal diet thus prepared was estimated to contain 1.7 μ g of biotin per 100 g of diet.

A 100 g portion of the diet was thoroughly mixed with a little amount of water, dried at room temperature under reduced pressure, and was crushed into scrambles. The diet was freshly prepared every 10 days, sealed in bottles with nitrogen, and stored in a refrigerator until needed.

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Carp weighing 3.6 g in average were divided into 6 groups of each 50 fish and kept in aquariums (30×30×50 cm) supplied with a constant flow of city water dechlorinated by activated charcoal and maintained at 20°C. They were subjected to disinfect with malachite green, acromycin and acriflavine before feeding trial. The fish were fed for 80 days, 6 days weekly, on a rigid schedule and body weight was estimated at an interval of 10 days.

Determination of biotin and crude fat The vitamin was determined microbiologically by using *Lactobacillus arabinosus*¹¹⁾, and crude fat by the method of FOLCH *et al.*¹²⁾

Examination of histopathology At the end of the feeding trial, samples of each 5 fish from the biotin-deficient and the biotin supplemented (0.6 mg %) groups were prepared for histopathological examination. The tissues of samples were fixed with Zenker-formol solution and stained with Mayer's acid hemalum-eosin (HE), Heidenhain's azo-carmin blue-orange G(AZAN) and periodic acid-Schiff reagent(PAS). Additional 10–13 fish were used for the examination of hematology.¹⁵⁾

Results and Discussion

Growth and requirement The growth responses of the fish to the dietary levels of biotin are summarized in Table 1 and Fig. 1. In each group, no dead fish was observed throughout the experimental period. There was no significant difference in weight gain among the groups supplemented with biotin (groups 2 to 6). The fish fed the deficient

Table 1. The dietary levels of biotin and growth of carp.

Group	Biotin added	Average body weight		Weight gain
		Initial	Final	
1	mg/100 g diet 0*	g 3.6	g 13.0	% 362
2	0.05	"	15.5	431
3	0.1	"	16.6	461
4	0.3	"	16.8	467
5	0.6	"	18.0	500
6	1.0	3.5	16.7	478

* Basal diet contained 1.7 µg of biotin per 100 g.

diet in biotin (group 1) appeared normal for first 60 days except a slight decrease in food consumption. During next 20 days, there was reduction in the intake of food, accompanied by decreased activity and weight gain, indicating that biotin is an indispensable dietary factor for growth of young carp. In the present study, however, the detectable abnormality such as "blue slime" disease observed on the brown trout²⁾ could not be seen among the fish fed a biotin-deficient diet. After 80 day-feeding, the fish of each group

were analyzed for biotin content of the hepatopancreas and muscle respectively. The analytical data are presented in Table 2. The biotin contents in the hepatopancreas of the fish fed the diets containing over 0.1 mg % of biotin were kept at nearly constant

Fig. 1. Dietary biotin levels and the growth responses of carp.
Numbers in figure indicate the experimental groups shown in Table 1.

level, whereas in the fish fed the diets containing below 0.05 mg % of biotin the contents were considerably dropped. The relation between biotin contents of the hepatopancreas and of the diets is shown in Fig. 2. The maximum storage of biotin in the hepatopancreas was found to attain when the diet contained near 0.1 mg of biotin per 100 g of diet. The

Fig. 2. Dietary biotin levels and biotin contents in hepatopancreas of the carp after 80 days feeding.

Table 2. Dietary biotin levels and the contents of biotin and lipids in hepatopancreas and muscle after 80 days feeding.

Group	Biotin added mg/100 g diet	Biotin content		Crude fat	
		Hepatopancreas	Muscle	Hepatopancreas	Muscle
1	0	0.42	0.006	5.6	1.5
2	0.05	0.53	0.029	9.2	1.5
3	0.1	0.87	0.051		
4	0.3	0.88	0.061		
5	0.6	0.91	0.092	7.3	1.6
6	1.0	0.78	0.128	7.0	1.3

growth response of the fish under this dietary condition is also satisfactory. Quantitative requirement of young carp for biotin, therefore, is estimated to be near 0.1 mg in 100 g of diet under the experimental conditions adopted or 0.02–0.03 mg per kg of body weight per day because the diet was given in average at the rate of 2.5% of body weight. The value obtained in the present study was generally agreeable with the requirement of the fingerling chinook salmon (0.03–0.04 mg/kg weight/day).³⁾ The biotin content of the muscle increased in a proportion to the vitamin content in the diets. Several investigators^{13,14)} reported that the excessive amount of biotin in diet produced fatty liver in rat. In the present study, as shown in Table 2, there was no relationship between biotin intake and lipid contents of the tissues.

Histopathology Among the organs tested, distinctive morphological changes of the tissues from the fish of group 1 were observed on pancreas, kidney, gill, skin and lateral muscle respectively (Fig. 3). The pancreas showed atrophy of acinous cells and was frequently recognized the presence of vacuoles containing colloidal substances stained blue with AZAN but negative with PAS (Fig. 3–2).

The epithelial cells of urinary tubes contained a large number of glycogen-like granules which were PAS positive (Fig. 3–4). For the gill, the short-thickened gill lamellae accompanied by narrow capillary lumen and hypertrophy of the respiratory epithelial cells were noticed (Fig. 3–6). HALVER³⁾ reported that the chinook salmon fingerling fed a biotin-deficient diet developed muscle atrophy. In the present study with carp, however, such regressive degeneration of the muscle tissue could not be observed. But the increase of connective tissue and considerable amounts of glycogen-like deposition which were PAS positive in internal perimysium were conspicuous changes as compared with the control fish (Fig. 3–8).

The increase of mucous cells and their PAS reaction and little disarrangement of germinal cells were noticeable changes in the skin. These observations for skin may have some connection with the blue slime disease of brown trout.

Hematology The results of hematological examination is summarized in Table 3.

Fig. 3. The characteristics of histological patterns of carp fed biotin-deficient diet.

1. Acinous cells of pancreas of normal carp.
2. Acinous cells of pancreas of the deficient carp, showing atrophy and the presence of vacuoles containing colloidal substances.
3. The epithelial cells of urinary tubes of normal carp.
4. The epithelial cells of urinary tubes of the deficient carp, containing a large number of glycogen-like granules.
5. The gill of normal carp.
6. The gill of the deficient carp, showing the short-thickened gill lamellae accompanied by narrow capillary lumen and hypertrophy of the respiratory epithelial cells.
7. Lateral muscle of normal carp.
8. Lateral muscle of the deficient carp, showing the increase of connective tissue and considerable amounts of glycogen-like deposition in internal perimysium.

Table 3. Results of hematological examination.

	Group	Fish number	Mean $\pm$ SD*	Maximum	Minimum	Significance**
Erythrocyte count ($10^6/\text{mm}^3$)	5	13	1.93 ± 0.25	2.31	1.58	<0.05
	1	12	1.80 ± 0.39	2.56	1.27	
Immature erythrocyte (%)	5	12	1.16 ± 0.62	2.48	0.19	<0.001
	1	10	3.03 ± 0.85	4.46	2.07	
Leucocyte count ($10^4/\text{mm}^3$)	5	12	1.83 ± 1.22	5.02	0.58	<0.01
	1	10	2.90 ± 1.91	4.50	0.36	

* Mean $\pm$ Standard Deviation

** By t-test

The marked changes recognized with blood from the deficient fish were the increase of leucocyte and immature erythrocyte counts. No statistical difference was observed in the erythrocyte counts between the fish of control and lack.

Summary

- 1) The young carp were reared with the diets containing grading amounts of biotin.
- 2) The fish fed a biotin-deficient diet showed poor growth and a diminution of biotin content in the hepatopancreas.
- 3) Nutritional requirement of young carp for biotin was 0.1 mg per 100 g of diet or 0.02–0.03 mg per kg of weight per day.
- 4) The changes in histological and hematological analyses were also recognized on the biotin-deficient fish.

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