Water-soluble Vitamin Requirements of Carp—I.
Requirement for Vitamin B₂

Hiroshi AOE, Isao MASUDA, Takashi SAIITO, and Atuko KOMO*
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The requirements and deficiency symptoms of both trout and salmon for water-soluble vitamins have been extensively studied by the American workers. Knowledge on carp, *Cyprinus carpio*, however, is still lacking, although carp is one of the most important fresh-water fishes produced in Japan.

HASHIMOTO¹ suggested the possible bacterial production of vitamin B₁₂ in the digestive tract and OGINO² has recently established the deficiency symptoms and requirement of vitamin B₆.

To provide the sufficient basis for formulating diet, nutritional requirements of carp especially for B vitamins were studied by using the basal diet devised by HALVER et al.³ The present paper deals with deficiency symptoms and requirement of vitamin B₂ of the young carp.

Experimental and Results

**Diet.** The test diet devised by HALVER et al.³ for estimation of vitamin requirements of trout was adopted after a slight modification. It consisted of casein 54, gelatin 15, soy-bean oil 9, white dextrin 8, mixed minerals 4, DL-methionine 1, L-tryptophan 0.5, celluflour plus crystalline vitamins 9. Corn oil in the original formula was substituted by refined soy-bean oil. The commercially available, vitamin-free casein was washed with hot 70% methanol completely to remove vitamin B₂ contained in it at the level of 0.025 mg %. Gelatin used was a vitamin-free preparation from DIFCO Co.

A 100 g portion of the diet was mixed with 150 ml of warm water at 40°-50°C, and frozen in a freezer at −18° to −25°C. The firmness increased on freezing and the diet could be easily fed without wastage by the fish. Only as much diet as needed for one week feeding was prepared, stored in a freezer, and granulated on feeding.

**Determination of vitamin B₂.** Concentration of riboflavin in diet or experimental fish was determined chemically by the method utilizing the fluorescence of lumiflavin⁴.

**Methods.** Five plastic troughs of each 100 l capacity were used. They were supplied at the rate of 1 l/min. with well water adjusted at 22°-25°C and aerated sufficiently.

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The fish were fed four to five times everyday, and at the level of approximately 10% of the total body weight. The quantities of diets were adjusted every week on weighing the experimental fish. Dead or obviously moribund fish were removed as soon as observed and stored immediately after gross observation in 10% formalin solution for histological investigation.

**A preliminary test on vitamin B<sub>2</sub> requirement.** To see the deficiency symptoms and approximate level of requirement, young carp were kept on diets containing different quantities of vitamin B<sub>2</sub>.

After being fed preliminarily on the complete basal diet for 2 weeks, young carp weighing 1.5 g were divided into five groups of each 40 fish and fed on the experimental diet for 8 weeks, as shown in Table 1. Fish in Group 2 received vitamin B<sub>2</sub> from the unpurified casein used as a protein source. Fish in the other groups received different quantities of riboflavin incorporated into the basal diet consisting of the methanol-washed, vitamin-free casein and the vitamin mixture from which vitamin B<sub>2</sub> was eliminated. This experiment started on July 19 and terminated on September 29.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Vitamin B&lt;sub&gt;2&lt;/sub&gt; in diet mg</th>
<th>Vitamin B&lt;sub&gt;2&lt;/sub&gt; intake mg/kg body weight/day</th>
<th>Average body weight g 4th week</th>
<th>Average body weight g 8th week</th>
<th>Mortality %</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3.2</td>
<td>-</td>
<td>80</td>
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<tr>
<td>2</td>
<td>0.015</td>
<td>0.006</td>
<td>3.1</td>
<td>-</td>
<td>40</td>
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<tr>
<td>3</td>
<td>0.2</td>
<td>0.06</td>
<td>4.2</td>
<td>8.7</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>0.59</td>
<td>4.8</td>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>20.0</td>
<td>6.46</td>
<td>4.4</td>
<td>11.8</td>
<td>0</td>
</tr>
</tbody>
</table>

The results are summarized in Table 1 and Fig. 1. Absence or deficiency of vitamin B<sub>2</sub> is reflected by the poor growth. Fish of Groups 1 and 2 do not show any growth after the second week and the retardation of growth in Group 3 is significant at the end of experiment.

Deficiency symptoms were most evidently manifested by fish in Groups 1 and 2. In these groups, anorexia was observed after three weeks, and the fish floated on the surface responding to the sound on feeding, but would not catch the diet skilfully. The fish then became extremely thin and showed the high mortality. Hemorrhagic or damaged epidermis was also observed on the fourth week. Almost of all fish in Group 1 and nearly 80% of fish in Group 2 were extremely thin.
with or without damaged epidermis on the sixth week. In Group 3, however, no apparent symptoms were recognized except retardation of growth.

In histological investigation, intra-myocardial hemorrhage in auricle and ventricle was remarkable, especially in the latter. The section of myocardia of a dead fish in Group 1 on the fourth week is shown in Fig. 2, in comparison with that of normal fish. In the anterior part of the kidney, atrophy and partial necrosis of urinary tubule were apparent, and in the posterior part, karyopyknosis, karyolysis, and partial necrosis in the epithelium of urinary tubule were recognized, as seen in Fig. 2. In the figure, the kidney of a dead fish in Group 1 on the fourth week is shown. Moreover, atrophy and edema were noticeable in the muscle, and stripping, partial necrosis, and lymphoid cell infiltration were conspicuous in the stomach epithelium.

The surviving fish in Group 1 were divided into halves for recovery test at the

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Fig. 2. The myocardia and kidney of normal and vitamin B2-deficient carp (×280).
1. Myocardia of normal carp.
5th week and those in Group 2 at the end of 7th week. One half was fed the complete diet and the other kept continuously on the deficient or low diet. When the missing vitamin was supplied to the diets, fish gradually recovered as shown in Fig. 3, although at the beginning some fish died in succession.

Estimation of requirement of vitamin B₂. The quantitative requirement of vitamin B₂ was estimated from body weight gain, vitamin concentration in the hepatopancreas and feed conversion at levels, 0.1, 0.2, 0.4, 0.8 and 2.0 mg % of vitamin B₂.

The procedures were quite similar to those described above. The fish used were obtained from Groups 3, 4 and 5 at the end of previous experiment and shortage of fish was supplemented from the stock which had been maintained on the complete diet in parallel. Five groups of each 20 carp weighing 12.8 g in average were placed in troughs from October 5 for 6 weeks. At the end of experiment, 8-14 fish were taken from each group, and the hepatopancreas was analyzed for vitamin B₂ on the pooled sample. During the experimental period, fish of each group were healthy and not dead at all. The results are shown in Table 2 and Fig. 4. The relationships of vitamin B₂ concentration in diet to body weight gain, feed conversion on wet basis, and vitamin B₂ concentration in the hepatopancreas are shown in Fig. 5.

Using the method of least squares, equations for each straight rising line at the lower level of vitamin B₂ were determined, and intersecting points with the horizontal lines at the higher level were sought. The same value was obtained for both items, weight gain and feed conversion. The values obtained were 0.11 mg per kg of body weight per day or 0.40 mg per 100 g of diet according to body weight gain. The corresponding values were 0.17 mg or 0.62 mg according to storage level in the

| Table 2. Results of the experiment to estimate the requirement of vitamin B₂ |
|-----------------------------|-----------------------------|------------------------|------------------------|-----------------------------|
| Group No. | Vitamin B₂ added mg % | Vitamin B₂ intake mg/kg body weight/day | Average body weight gain g | Feed conversion % | Vitamin B₂ in hepatopancreas mg % |
|-------|----------------|-----------------|-----------------------------|-----------------------------|
| 6    | 0.1            | 0.025           | 22.0                        | 36.2                        | 0.47 ± 0.05                  |
| 7    | 0.2            | 0.050           | 29.6                        | 43.4                        | 0.63 ± 0.08                  |
| 8    | 0.4            | 0.109           | 37.2                        | 47.8                        | 1.21 ± 0.11                  |
| 9    | 0.8            | 0.212           | 35.3                        | 47.1                        | 1.79 ± 0.11                  |
| 10   | 2.0            | 0.564           | 39.8                        | 45.6                        | 1.73 ± 0.18                  |
hepatopancreas, respectively.

**Fig. 4.** Increase in average body weight.

**Fig. 5.** The relationships of vitamin B2 concentration in diet to average body weight gain, feed conversion, and vitamin B2 concentration in hepatopancreas.


**Discussion**

Elimination of vitamin B2 from the complete test diet devised by HALVER et al. caused typical deficiency symptoms in young carp, such as anorexia, poor growth, high mortality, and hemorrhages in epidermis and hepatopancreas. Recovery test indicated that some of these syndromes apparently depend on deficiency of vitamin B2.

MCLAREN et al.⁵ observed poor growth and hemorrhages in the eye, nose and operculum as vitamin B2 deficiency syndromes in rainbow trout. Beside these, HALVER⁶ recorded on chinook salmon corneal vascularization, cloudy lens, hemorrhagic eyes, photophobia, dim vision, incoordination, abnormal pigmentation of iris, striated constrictions of abdominal wall, dark coloration and poor appetite. In silver salmon, COATES et al.⁷ described a slight clouding of the eye lens which appeared in some of the moribund fish late in the experiment. PHILLIPS et al.⁸ reported stopped growth and clouding of the eyes lens in trout and salmon. In carp such abnormalities in the eye lens and dark coloration were not recognized.

The vitamin B2 requirement per kg of body weight per day of rainbow trout were reported to be 0.44–0.68 mg by PHILLIPS⁸⁻¹³, 0.1–0.3 mg by MCLAREN⁵, and for chinook salmon 0.75–1.20 mg by HALVER⁴⁰. As the requirements depend on the various factors, such as the size of fish, water temperature, movement of fish and other constituents of diet, it should be better to avoid the detailed discussion on the difference between salmonoid fish and carp. The similar values, however, were obtained on the young carp in this experiment. It may be note-worthy that the value by hepatopancreas storage levels seems to be higher also in rainbow trout than that by the growth rate.
Summary

1. The deficiency symptoms and requirement of vitamin B₂ of young carp have been studied, using the basal diet devised by HALVER et al.

2. The deficiency symptoms were characterized by anorexia, poor growth, high mortality, hemorrhages in both epidermis and hepatopancreas. These symptoms were alleviated by administration of vitamin B₂.

3. Under the experimental conditions adopted, the requirement of young carp was found to be 0.11 mg per kg of body weight per day (0.40 mg per 100 g of diet) on the basis of body weight gain, and 0.17 mg per kg of body weight per day (0.62 mg per 100 g of diet) on the basis of storage levels of vitamin B₂ in the hepatopancreas.

Acknowledgement

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References

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2) C. OGINO: This Bull., 31, 546~551 (1965).