FINFISH NUTRITION IN ASIA
Methodological Approaches to Research and Development

C.Y. Cho, C.B. Cowey, and T. Watanabe
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Finfish nutrition in Asia: methodological approaches to research and development. Ottawa, Ont., IDRC, 1985. 154 p.: ill.

UDC: 597:591.13

Microfiche edition available
FINFISH NUTRITION IN ASIA
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(Includes Proceedings of the Asian Finfish Nutrition Workshop held in Singapore, 23–26 August 1983)

C.Y. Cho, C.B. Cowey, and T. Watanabe
Abstract

Fish nutrition is fundamental to most aquaculture practices in Asia. Although IDRC research support aims, in part, to promote the culture of species requiring no supplementary feeding, it is recognized that formulated feeds are required to increase productivity of many important species now being cultured within the region. This requirement will undoubtedly continue in the future.

Through observing some nutrition research projects, it has become apparent that many of the basic approaches to applied nutrition are not readily available to researchers. Thus, part I of this publication deals with methodological approaches to research and development. Included are discussions on nutrient requirements and deficiencies, fish feeds and their quality, feeding practices, nutrition of broodstock and larvae, and approach and design of nutrition experimentation, as well as an extensive reference and suggested reading list.

Part II presents the proceedings of the Asian finfish nutrition workshop held in Singapore, 23–26 August 1983. Included are research papers dealing with a variety of questions that are important for countries within the region.

Résumé

La nutrition des poissons est une question primordiale pour la plupart des exploitations d’aquiculture en Asie. Le CRDI subventionne des recherches destinées à favoriser la culture d’espèces n’ayant pas besoin d’un supplément alimentaire. Il est cependant conscient que la nourriture commerciale est nécessaire à l’augmentation et au maintien de la productivité de nombre d’espèces cultivées en cette région.

L’observation de certains projets de recherche en nutrition a révélé que plusieurs approches fondamentales de la nutrition appliquée ne sont pas facilement accessibles aux chercheurs. La première partie de la publication porte donc sur les approches méthodologiques de la recherche et du développement. Elle aborde notamment les besoins et les carences en matières nutritives des poissons, leur nourriture et sa qualité, les méthodes d’alimentation, la nutrition des géniteurs et des larves, de même que l’optique et la méthode à appliquer à la confection des expériences de nutrition. Elle comprend aussi une importante liste des références et de lectures suggérées.

La deuxième partie, les actes de l’atelier sur la nutrition des poissons qui s’est tenu à Singapour du 23 au 26 août 1983, reproduit des documents de recherche portant sur une gamme de questions importantes pour les pays de la région.

Resumen

La nutrición de los peces es elemento fundamental en la mayoría de las prácticas de acuacultivo en Asia. A pesar de que la ayuda que brinda el CIID a las investigaciones está destinada, en parte, a fomentar la cria de especies que no requieran alimentación complementaria, se admite la necesidad de suministrar piensos formulados para aumentar la productividad de muchas especies importantes que se crían ahora en esta región. Todo parece indicar que esta situación persistirá en el futuro.

El análisis de algunos proyectos de investigaciones sobre nutrición ha revelado que los investigadores no tienen fácil acceso a muchos enfoques básicos en el campo de la nutrición aplicada. Es por esto que en la primera parte de esta publicación se analizan enfoques metodológicos relativos a las actividades de investigación y desarrollo. Los temas tratados en la primera parte incluyen: necesidad y carencia de nutrientes; piensos para peces y la calidad de los mismos; prácticas de alimentación; nutrición de las crías y larvas; y principios y diseño de experimentos sobre nutrición. También se incluye una extensa lista de referencias y de lecturas complementarias.

La segunda parte recoge los debates del seminario sobre nutrición de los peces en Asia, celebrado en Singapur del 23 al 26 de agosto de 1983. Se incluyen algunas ponencias que tratan de muchas cuestiones de importancia para los países de la región.
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Water-Soluble Vitamins Essential for the Growth of Clarias

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Catfish fingerlings (Clarias batrachus Linn.) were fed eight test diets: diet 1, the control, was a complete vitamin diet; diets 2–8 were devoid of water-soluble vitamins B1 (thiamine), B2 (riboflavin), B6 (pyridoxine), pantothenic acid, folic acid, niacin, and vitamin C (ascorbic acid) respectively. Average weight gains, growth rates, and the effect of the vitamin-deficient diets were then observed.

Introduction

The walking catfish (Clarias batrachus Linn.) is native to Southeast Asia, occurring in rivers, canals, lakes, swamps, and flooded marshes or fields. Clarias is one of the most economically important cultured species in Thailand. The fish can tolerate poor water quality, is adaptable to high stocking density, eats a wide range of supplemental feeds, and is able to spawn naturally in captivity. The problem that farmers are currently facing, however, is high mortality. This might be due to stress susceptibility induced by inbreeding or malnutrition.

The increasing importance of this species in commercial fisheries demands considerable research on its nutritional requirements. Water-soluble vitamins have been identified as being essential for salmon and trout (Halver 1957; Coates and Halver 1958; Kitamura et al. 1967; Phillips et al. 1955), channel catfish (Dupree 1966), carp (Aoe et al. 1967, 1969), and eels (Arai et al. 1972). No research on the vitamin requirements of Clarias has been reported. The present study, therefore, was designed to ascertain the specific water-soluble vitamin requirements of Clarias on the basis of growth and mortality.

Materials and Methods

Facilities and Fish

Thirty-two 50 cm × 90 cm × 50 cm glass aquaria, located in a wet laboratory at the National Inland Fisheries Institute, were used in a 24-week feeding experiment. The well water in each aquarium was aerated and changed every other day. Clarias fingerlings, obtained from a private hatchery in Chacheongsao Province, were used in the study. Initially, the fingerlings weighed 4–5 g, with an approximate length of 7–8 cm. They were stocked in August 1982 at a density of 12 fish per aquarium.
Test Diets

A semipurified complete diet was used as the control diet. The composition of the complete diet, including vitamins and minerals, is presented in Table 1. Thiamine, riboflavin, pyridoxine, pantothenic acid, folic acid, niacin, and ascorbic acid were individually deleted from the complete diet to form seven test diets.

The dry ingredients of each diet were mixed in a Hobart mixer for 6-7 min, then 6% oil and 30% water were added and mixed. The moist diet mixture was passed through a 1.6-mm diameter die in a food grinder. The spaghetti-like diet was then broken into 1.0-1.5 cm length pellets and kept in the freezer until it was fed to the fish.

Management

Fish from the farm were stocked in a fibreglass tank, checked, and treated for parasites and diseases. They were then trained to feed on pellets for 3 weeks. Prior to starting on the test diets, the fish were stocked in the aquaria and fed a complete diet for 1 week. The aquaria were randomly chosen to receive either the complete vitamin diet or one of the seven vitamin-deficient diets. Each diet was fed to four replicate aquaria. The fish were fed twice daily, 0900 hours and 1600 hours, 6 days per week. The feeding rate was 10% of the body weight per day. The fish were sampled triweekly for weight measurements and observed for gross signs of vitamin deficiency. The feed allowance was adjusted subsequent to weight measurement. The fish were fed the test diets for 24 weeks. At the end of 12 weeks, after vitamin-deficiency symptoms had developed, two of the four aquaria receiving vitamin-deficient diets began receiving the complete diet.

The fish were observed twice daily at feeding time and a close postmortem examination was made during each weighing period every 3 weeks. A gross postmortem examination was made of the liver, kidneys, stomach, and intestines. No histological examination was made. The growth data were subjected to analyses of variance (Steel and Torrie 1960).

Table 1. Composition of the complete vitamin test diet for Clarias.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein, vitamin free</td>
<td>29.0</td>
</tr>
<tr>
<td>Corn dextrin</td>
<td>30.0</td>
</tr>
<tr>
<td>Salad oil</td>
<td>6.0</td>
</tr>
<tr>
<td>Vitamin mix&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0</td>
</tr>
<tr>
<td>Mineral mix&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>18.5</td>
</tr>
<tr>
<td>Carboxymethylcellulose</td>
<td>3.0</td>
</tr>
<tr>
<td>Gelatin</td>
<td>6.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Vitamin mix (mg/kg diet): vitamin A, 3; vitamin D, 0.05; vitamin E, 50; vitamin K, 10; choline, 550; niacin, 100; riboflavin, 20; pyridoxine, 20; thiamine, 20; D-calcium pantothenate, 50; biotin, 0.1; folic acid, 5; vitamin B<sub>12</sub>, 0.002; vitamin C, 100; inositol, 100.

<sup>b</sup> Mineral mix (g/kg diet): CaHPO<sub>4</sub>·2H<sub>2</sub>O, 2.07; CaCO<sub>3</sub>, 1.47; KH<sub>2</sub>PO<sub>4</sub>, 1.0; NaCl, 0.6; MnSO<sub>4</sub>·H<sub>2</sub>O, 0.015; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.05; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.30; KIO<sub>3</sub>, 0.001; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.003; ZnCO<sub>3</sub>, 0.015; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.00017; NaMoO<sub>4</sub>·2H<sub>2</sub>O, 0.00083; Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, 0.00002.
Results and Discussion

Complete Vitamin Diet

*Clarias* receiving the complete diet exhibited weight gains averaging from 4.70 to 36.75 g during the first 12 weeks and 34.82 to 62.6 g during the second 12 weeks. Abnormality in morphology and behaviour was not observed, nor was any mortality during the 24-week period.

Thiamine-Deficient Diet

The results of feeding thiamine-deficient and complete vitamin diets are illustrated in Fig. 1. No significant differences in weight gain or mortality were demonstrated, nor did postperiod examination of the fish show any abnormal condition in the liver, kidneys, stomach, or intestines. The only abnormality observed in the fish was a dark skin colour.

Riboflavin-Deficient Diet

Results illustrated in Fig. 2 show no significant difference in average weight gain. The differences in cumulative mortality between the riboflavin-deficient group and the complete vitamin group at the end of 12 weeks and 24 weeks were highly significant. In fish fed the riboflavin-deficient diet, deaths began to occur after 5 weeks, followed by a reduction in food consumption and activity. Food consumption resumed, however, by week 12.

Examination of the riboflavin-deficient fish revealed fragile fins; hemorrhaging under the skin, at the fins, and around the eyes; eroded barbels; edema; fading of body colour; poor appetite; lethargy; pale gills; pale liver; and slight opaque lens at the end of the experiment. The recovery test initiated after 12 weeks of feeding on the B2-deficient diet indicated a significant decrease in mortality and improvement in deficiency symptoms and growth.

Pyridoxine-Deficient Diet

The results of utilizing this diet are illustrated in Fig. 3. The diet significantly retarded growth after 3 weeks. Death and deficiency symptoms developed after 8 weeks. The group showed mortality rates of 38.8 and 88.8% at 12 and 24 weeks, respectively, which was highly significant compared with the group fed the complete diet.

B6-deficiency symptoms in *Clarias* involved eroded barbels, tetany, nervous disorders, loss of equilibrium, and erratic swimming habits such as whirling, twisting, and swimming in circles. Prior to dying, the fish surfaced frequently, floating on the surface or sometimes sinking to the bottom immediately, where they lay still and experienced rapid breathing. Gross postmortem examinations revealed eroded fins and lower jaw. The internal organs, however, including the stomach, liver, kidneys, intestine, and gill, appeared normal. Mortality and deficiency symptoms were eliminated after feeding on the complete diet for 3 weeks.
Fig. 1. Comparison of the average weight gains and cumulative mortalities of Clarias fed thiamine-deficient and complete vitamin diets.
Fig. 2. Comparison of the average weight gains and cumulative mortalities of Clarias fed riboflavin-deficient and complete vitamin diets.
Fig. 3. Comparison of average weight gains and cumulative mortalities of Clarias fed pyridoxine-deficient and complete vitamin diets.
Pantothenic Acid Deficient Diet

Use of this diet resulted in significant differences in average weight gain after only 3 weeks of feeding (Fig. 4). There was an extreme reduction in food intake, accompanied by decreased activity and weight gain during weeks 9–12. Mortality occurred in week 6. Prior to death, the fish were injured by rubbing against the bottom of the aquarium, causing lesions and necrosis. Examination of the fish revealed clubbed gills, hemorrhaging under the skin, fragile fins, edema, eroded barbels, rapid breathing, and swelling at the base of the pectoral fins.

As the experiment progressed, a greater number of fish demonstrated these symptoms and cumulative mortality reached 100% at the end of the experiment. After supplementation with pantothenic acid in the test diet, recovery was slow, although mortality ceased and deficiency signs disappeared after 3 weeks. Growth, however, improved only after 9 weeks on the recovery diet.

Gross postmortem examination revealed pale gills and livers. As well, the gills were covered with excessive mucous. The fish showed high concentrations of fat in the abdominal cavity and pits at the anterior part of the body just behind the skull on both sides of the occipital process.

Folic Acid Deficient Diet

The results of using this diet are illustrated in Fig. 5. The differences in weight gain and cumulative mortality between the folic acid deficient group and the complete vitamin group were significant at the end of 6 and 12 weeks respectively. Fish fed the deficient diet appeared normal, except for a slight decrease in food consumption accompanied by decreasing weight gain. Gross postmortem examination revealed fading of body colour, pale gills, and pale liver.

Niacin-Deficient Diet

The results of feeding this diet are presented in Fig. 6. The average weight gain and cumulative mortality of fish fed the niacin-deficient and complete diets were significantly different at the end of 12 and 24 weeks respectively. Loss of appetite was the first sign noted. Upon continued exposure to the deficient diet, muscle spasms, loss of equilibrium, whirling, lethargy, hemorrhaging under the skin and fins, and slightly protruding eyes were observed. Prior to death, the fish darted to the surface and immediately sank to the bottom, with some fish experiencing convulsions. After niacin was added to the diet, mortality ceased and the fish began to eat and grow normally again.

Ascorbic Acid Deficient Diet

The results of feeding this diet are illustrated in Fig. 7. There were no significant differences in weight gain or mortality between fish fed the ascorbic acid deficient diet and those on the control diet. Cumulative mortality in the deficient group was 16.66% and in the control group was 0% at the end of 24 weeks. However, the fish fed the ascorbic acid deficient diet showed scoliosis, external hemorrhaging, fin erosion, and dark skin colour at 12 weeks. Upon examination at the termination of the experiment, no abnormal features were noted in the gills, stomach, liver, kidneys, or intestines.
Fig. 4. Comparison of average weight gains and cumulative mortalities of Clarias fed pantothenic acid deficient and complete vitamin diets.
Fig. 5. Comparison of average weight gains and cumulative mortalities of Clarias fed folic acid deficient and complete vitamin diets.
Fig. 6. Comparison of average weight gains and cumulative mortalities of Clarias fed niacin-deficient and complete vitamin diets.
Fig. 7. Comparison of average weight gains and cumulative mortalities of Clarias fed ascorbic acid deficient and complete vitamin diets.
Summary

The deficiency symptoms of Clarias fed thiamine-deficient diets were not as severe as those observed in salmon or trout. This might be a result of the availability of B$_1$ produced by intestinal microorganisms. Riboflavin-deficiency symptoms in Clarias were similar to those in salmon, trout, and channel catfish, except the former exhibited normal growth and fading of body colour, whereas the others showed poor growth and dark skin colour. Pyridoxine was essential for both growth and survival of Clarias. The pantothenic acid deficient diet developed the most severe deficiency symptoms, very poor growth and eventual fatality. The swelling at the base of the pectoral fins, presumably caused by bacterial infection induced by stress due to the pantothenic acid deficiency, disappeared with time as the fish became more mature. Because folic acid is required for normal blood formation, deficiency of this vitamin caused anemia and decreased weight gain and survival in Clarias as well as in trout, salmon, and channel catfish. Some niacin-deficiency symptoms in Clarias, such as protruding eyes, were different from those observed in other species of fish. Lesions on the isthmus were one of the distinctive signs of vitamin-C deficiency in Clarias (Boonyaratpalin et al. 1982), but were not found in this experiment. This is probably a result of the differences in experimental conditions, age of fish, or amount of food intake.