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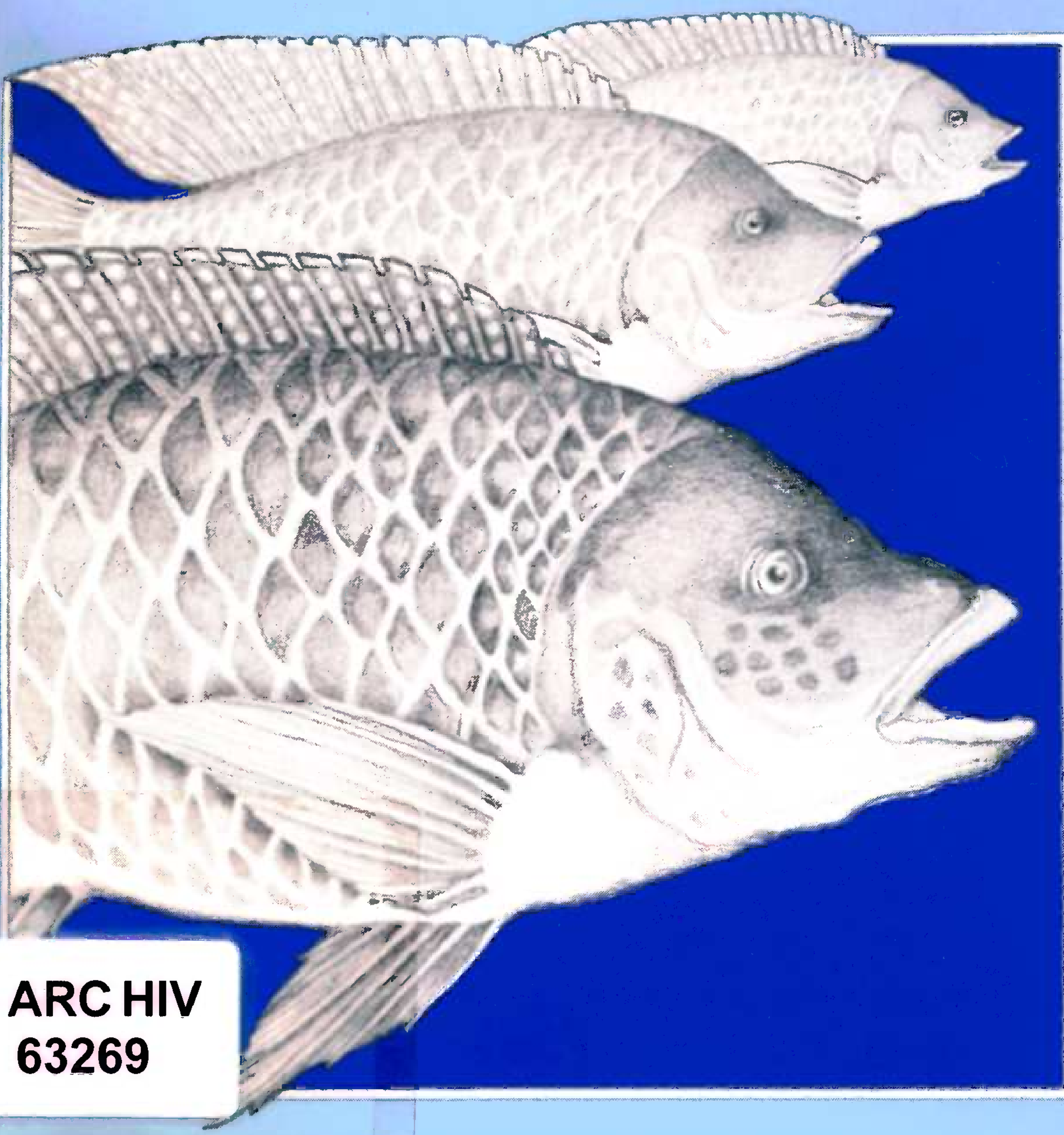
FINFISH NUTRITION IN ASIA

Methodological Approaches to Research and Development

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



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FINFISH NUTRITION IN ASIA
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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 ce moment dans la 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 conception 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 mayoria 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 cría 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 crian 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 crias 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 paises de la región.

Contents

Lipid Composition of Milkfish Grown in Ponds by Traditional Aquaculture

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Milkfish is the most important cultured food fish in the Philippines, Taiwan, and Indonesia. Traditional milkfish aquaculture in the Philippines depends upon two natural food bases: one consists of a complex of unicellular algae and diatoms; the other consists of filamentous green algae, predominantly *Chaetomorpha brachygona*. The lipid composition of milkfish reared on a unicellular algal complex (sample A) and on filamentous algae (sample B) was determined. The fatty-acid pattern of the depot fat of both samples of milkfish reflected the diet. However, the fatty-acid composition of lipids from milkfish liver showed a marked increase in the levels of long-chain polyunsaturated fatty acids of the $\omega 3$ and $\omega 6$ series. This suggests that there was a metabolic transformation of dietary fatty acids in the liver. Although milkfish grown by traditional aquaculture feed on natural food that has relatively low lipid content, the fish actively metabolize and accumulate lipids through active metabolic transformation not only of available dietary lipids but also of the nonlipid nutrient components, such as carbohydrates and proteins.

Introduction

Milkfish is one of the most important food fish in Southeast Asia. It is widely cultured in Indonesia, the Philippines, and Taiwan (Chen 1976). In the Philippines, about 90% of the total aquaculture production comes from milkfish culture (BFAR 1976).

Traditional aquaculture techniques are still favoured by many Philippine fish farmers. These techniques rely on cultivation of natural food bases. As suitable areas for aquaculture become a limiting factor, an increase in productivity could be brought about by the use of artificial diets. Fundamental studies on nutrient requirements and metabolism are desirable to formulate artificial diets for aquaculture. Most fish are known to efficiently digest and metabolize lipids. However, there is no information on lipid metabolism, composition, and requirement of milkfish. This study, therefore, compares the lipid composition of milkfish grown on two different natural food bases by traditional aquaculture.

Materials and Methods

Fish Samples

Milkfish samples weighing about 200–300 g and grown on two types of natural food bases were collected from private fish ponds in Panay Island, Philippines. Each batch consisted of about 60 randomly sampled fish. After capture, the fish, packed in ice, were transported to the laboratory. The fish were dissected within 48 hours after capture.

Extraction of Total Lipids

The total lipids of individual livers and depot fat in the muscle of the belly section from each batch of fish and samples of natural food from the ponds where the fish were grown were extracted and purified using the method of Bligh and Dyer (1959). The iodine value of the total lipids was determined by the Wijs method as modified by Griffins (1968).

Lipid classes were resolved by column chromatography using silicic acid (Moerck and Ball 1973). Neutral lipids were fractionated further by column chromatography using florisil (Carroll 1961).

The total lipids were saponified and transesterified with 0.5 *N* methanolic sodium hydroxide and boron trifluoride-methanol reagent (Metcalfe et al. 1966). The fatty acid methyl esters were analyzed by gas–liquid chromatography on a Shimadzu GC-4C gas chromatograph using a flame ionization detector. The column was packed with 15% diethyleneglycol succinate (DEGS) on a 100-mesh acid-washed Chromosorb ω support. The column was made of stainless steel (3 m long, 3 mm i.d.) and the column temperature was set at 180°C. Chromatographic standards of polyunsaturated fatty acid methyl esters (No. 4-7033 and No. 4-7015) were purchased from Supelco, USA.

Results

Table 1 shows the physicochemical condition of the pond waters and the mean weight of the fish at the time of sampling. The fish samples were grown on two different types of natural food. Fish in sample A were grown on a community of unicellular algae and diatoms, whereas fish in sample B were fed fibrous filamentous green algae composed predominantly of *Chaetomorpha brachygona*. Both ponds had comparable values for parameters such as water temperature, pH, and dissolved oxygen but differed markedly in salinity. The salinity was higher in the pond from which fish in sample A were collected. The typical nutrient composition of the natural food bases is shown in Table 2.

Total Lipid Content and Iodine Number

Table 3 shows the total lipid content of the natural food, milkfish liver, and depot fat. The natural food contains very little lipid. The milkfish carcass has a lipid content of about 4.9% on a wet-weight basis. The depot fat generally contained more lipid than the liver. However, the total lipid content and iodine number for specific milkfish tissues were comparable. The iodine number of milkfish liver was higher than the lipids from the natural food.

Table 1. Pond conditions and other sampling data.

Parameter	Sample A	Sample B
Sampling date	28 August 1981	11 November 1981
Number of fish sampled	60	60
Mean weight (g) of fish ± standard deviation	250.03 ± 31.56	220.07 ± 37.79
Pond conditions		
Pond temperature	35.5°C	34.5°C
pH	8.3	8.4
Salinity[a]	59.86 ppt	13.40 ppt
Dissolved oxygen[b]	8.15 ppm O_2	10.10 ppm O_2
Nitrite-nitrogen	0	0.01 ppm N
Ammonia-nitrogen	0.10 ppm N	0.58 ppm N
Phosphate-phosphorus	0.17 ppm P	0.08 ppm P
Predominant natural food	Unicellular algal complex	Filamentous algae

[a] By titration method.
[b] By modified Winkler method (APHA 1976). Samples of pond water were not fixed.

Lipid Classes

Table 4 shows the results of fractionation of the total lipids by column chromatography using silicic acid. The total lipids were fractionated into three major classes: neutral lipid, phospholipid, and glycolipid.

The neutral lipid content of the unicellular algal complex was higher than that of the filamentous algae. The neutral lipid content of both the depot fat and liver of sample A milkfish was higher than that of sample B. On the other hand, the phospholipid content of the liver and depot fat of sample B was higher than that of sample A. The glycolipid content of the depot fat and liver from both milkfish samples was comparable. Milkfish liver has higher phospholipid and glycolipid contents than depot fat. The depot fat, however, contains more neutral lipids than the liver.

Neutral Lipids

Table 5 shows the results of fractionation of the neutral lipids into their various components by column chromatography using florisil. For milkfish tissues and natural food, the major neutral lipid components were the triglycerides, followed by cholesterol esters. The triglyceride content of the unicellular algal complex is higher than that of the filamentous algae. This trend is reflected in the higher content of triglycerides in both the liver and depot fat of sample A.

Table 2. Typical nutrient composition of natural algal food bases in milkfish aquaculture.

Nutrient component	Percentage dry-weight basis[a]	
	Sample A	Sample B
Crude protein	12.22	12.81
Crude fat	1.98	1.53
Crude fibre	7.46	10.44
Ash	38.83	33.31
Nitrogen-free extract	39.51	41.91

[a] The water content ranged from 78 to 82%.

Table 3. Percentage total lipid content and iodine number of natural food and milkfish tissues.

Sample	Percentage total lipid (wet-weight basis)	Iodine number
Natural food		
Unicellular algal complex	0.92	109.07
Filamentous algae	0.65	94.24
Milkfish depot fat		
Sample A	7.63 ± 1.72	138.13
Sample B	7.89 ± 1.92	136.91
Milkfish liver		
Sample A	2.91 ± 0.56	141.09
Sample B	2.66 ± 0.70	140.72

Fatty-Acid Composition

The fatty-acid composition of the total lipids from natural foods and milkfish tissues was analyzed by gas–liquid chromatography (Table 6). The natural food of the fish contained more saturated than unsaturated fatty acids. A similar trend was observed in the depot fat of both samples A and B. The milkfish liver, however, contained more unsaturated than saturated fatty acids. This trend is clearly reflected in the ratio of saturated to unsaturated fatty acids as well as in the total percentage of saturated and unsaturated fatty acids for the liver tissues analyzed.

In general, the fatty-acid pattern of the depot fat reflected that of the dietary fatty-acid composition. The fatty-acid composition of the liver, however, reflected both dietary patterns and metabolic transformations. Thus, the liver contains several long-chain polyunsaturated fatty acids that were not detected in the milkfish depot fat nor in the natural foods.

Discussion

Milkfish grown by traditional aquaculture feed on natural food that has relatively low lipid content. However, the fish actively metabolize and accumulate lipids from dietary lipids and other nutrients. The major depot fat was found in the muscle of the belly section. Similar to many other fish, milkfish depot fat has a higher lipid content than the liver. However, in other teleosts, such as cod, haddock and hake, practically all of the lipids are localized in their fairly large livers (Brody 1965). The neutral lipid fraction was the most predominant lipid class in milkfish tissues. This was

Table 4. Composition of lipid classes of milkfish tissues and its natural food.

Lipid classes	Natural food		Milkfish liver		Milkfish depot fat	
	A	B	A	B	A	B
Neutral lipid	50.87	46.20	66.87	58.78	78.98	74.35
Phospholipid	38.92	38.35	27.92	35.54	18.82	23.09
Glycolipid	10.18	15.35	5.21	5.66	2.19	2.56

Notes: Lipid classes were resolved by column chromatography as described in the materials and methods section. Classes are expressed as weight percentage of the total lipid.

Table 5. Percentage composition of the neutral lipid fraction of milkfish tissues and its natural food.

Neutral lipid classes	Natural food		Milkfish liver		Milkfish depot fat	
	A	B	A	B	A	B
Hydrocarbons	0.76	1.26	0.40	0.90	0.90	0.80
Cholesterol esters	11.96	16.78	8.50	11.24	12.36	13.86
Triglycerides	73.56	68.30	82.60	71.70	76.24	73.36
Cholesterol	3.60	1.86	1.80	2.70	2.38	2.12
Diglycerides	3.76	3.20	2.80	3.86	2.64	2.80
Monoglycerides	2.78	2.90	1.04	3.76	2.14	2.58
Free fatty acids	3.52	5.38	2.78	5.74	3.22	4.38

Notes: Neutral lipid fractions were resolved by column chromatography as described in the materials and methods section. Fractions are expressed as weight percentage of total neutral lipid.

Table 6. Percentage composition of the major acids in the lipids of milkfish tissues and its natural food.

Fatty acids	Natural food		Milkfish liver		Milkfish depot fat	
	A	B	A	B	A	B
10:0	2.00	0.25	—	—	—	—
12:0	1.14	0.94	1.09	1.14	0.56	0.86
14:0	10.36	9.04	2.07	2.19	4.15	3.30
15:0	2.65	2.83	1.92	1.53	7.22	5.78
16:0	20.72	26.69	14.18	23.59	22.86	31.18
16:1ω7	9.43	10.22	6.06	5.53	16.33	14.67
16:2ω4	6.26	5.86	—	—	1.06	1.65
17:0	6.50	6.41	1.14	1.72	3.59	3.67
17:1ω9	1.90	0.50	2.22	3.37	3.53	3.67
18:0	15.71	17.42	5.05	6.66	11.91	10.27
18:1ω9	15.36	16.56	9.17	20.30	21.83	17.61
18:2ω6	1.90	2.09	2.95	6.66	2.51	2.20
18:3ω3	4.06	0.31	3.45	3.21	1.44	1.10
20:1ω9	1.14	—	3.35	3.09	—	—
20:2ω6	—	—	6.77	1.11	—	—
20:3ω6	0.87	—	2.95	1.29	1.23	—
20:4ω6	—	0.10	4.04	1.74	—	0.43
20:4ω3	—	—	—	—	0.54	—
20:5ω3	—	—	19.60	4.64	—	—
22:1ω9	—	—	2.66	0.18	—	—
22:4ω6	—	—	7.84	8.72	—	0.24
22:5ω6	—	0.75	0.04	0.26	1.23	3.36
22:5ω3	—	—	3.45	3.06	—	—
Total ω3 FAs	4.06	0.31	26.50	10.91	1.98	1.10
Total ω6 FAs	2.77	2.94	24.59	19.78	4.97	6.23
Ratio ω3/ω6	1.47	0.10	1.08	0.55	0.40	0.18
Total saturated FAs	59.08	63.58	25.45	36.83	50.29	55.06
Total unsaturated FAs	40.92	36.39	74.55	63.16	49.70	44.93
Ratio saturated/ unsaturated FAs	1.44	1.74	0.34	0.58	1.01	1.22
Total monoenoic acids	27.83	27.28	23.46	32.47	41.69	35.95
Total polyunsaturated fatty acids	13.09	9.11	51.09	30.69	8.01	8.98

followed by phospholipids and then glycolipids. Earlier studies indicate that neutral lipids are the major constituent of fish lipids and that phospholipids and glycolipids are present in lesser amounts (Kanazawa et al. 1979). The neutral lipids of milkfish consist largely of triglycerides. The same observation was reported for a number of other fish by earlier investigators (Gruger et al. 1964; Lovern 1964; Cowey and Sargent 1979).

The fatty-acid pattern of milkfish depot fat reflected the dietary pattern. The depot fat of the fish contained more saturated than unsaturated fatty acids, a trend also observed in the natural food of the fish. The ratio of the total saturated to total unsaturated fatty acids, as well as the total percentage of polyunsaturated fatty acids (PUFAs), is comparable for both the natural food and depot fat. The results suggest that, in milkfish, dietary fatty acids are deposited essentially unchanged in their adipose tissues.

Previous workers have shown that the fatty-acid composition of depot lipids in fish is greatly influenced by diet. Freshwater eels (*Anguilla vulgaris*) fed exclusively with herring meal containing 20% of a marine type of lipid deposited this dietary component in a virtually unmodified pattern (Lovern 1938). The fatty-acid pattern of channel catfish (*Ictalurus punctatus*) fed three types of dietary lipids (beef tallow, safflower oil, and menhaden oil) was found to be similar to the dietary fatty-acid composition (Stickney and Andrews 1971). The statistical analysis of fatty-acid data on channel catfish grown on six experimental diets containing various sources and amounts of lipids showed that the fatty-acid composition of the fish was strongly influenced by the diet (Worthington and Lowell 1973).

The fatty-acid pattern of the milkfish liver tended to reflect not only the pattern of the dietary lipids but also fatty acids obtained through metabolic transformations of dietary lipids and other nonlipid nutrient components, such as carbohydrates and proteins. The fatty acids from the liver are much more unsaturated than the fatty acids from the depot fat and natural food. The increased unsaturation in the fatty acids from the liver is due to the presence of increased levels of long-chain PUFAs. Significant quantities of 20:2ω6, 20:3ω6, 20:4ω6, 20:5ω3, 22:4ω6, 22:5ω6, and 22:5ω3 fatty acids were found in the liver of both fish samples but not in the milkfish depot fat nor in the natural food. The presence of long-chain PUFAs in the liver, despite their absence in the natural food, suggests that chain elongation and desaturation have occurred and that the site of such metabolic transformations in milkfish is the liver.

The conversion of dietary fatty acids to long-chain PUFAs has been found to occur in fish (Mead et al. 1960; Kayama et al. 1963). Tracer studies on several species of fish, including rainbow trout, ayu, eel, red sea bream, rockfish, and globefish, also showed that by chain elongation and desaturation these fish are capable of converting exogenous 18:3ω3 to 18:4ω3, 20:3ω3, 20:4ω3, 20:5ω3, and 20:6ω3 (Kanazawa et al. 1979).

The mechanism for the synthesis and interconversion of fatty acids in fish is now well established and has shown that fish can synthesize de novo from acetate, fatty acids of the ω5, ω7, ω9, and ω11 series. Fish are unable, however, to synthesize fatty acids of the ω3 and ω6 series unless a precursor with this ω structure is present in the diet (Mead and Kayama 1967; Sinnhuber 1969). This limitation in biosynthetic capability accounts for the essentiality of ω3 and ω6 in most fish (Cowey and Sargent 1979; Castell 1979). The growth-enhancing effect of highly unsaturated ω3 fatty acid, particularly for marine fish, was demonstrated by Takeuchi and Watanabe

(1976, 1977).

The ability of milkfish to grow in natural food bases with relatively low lipid content reflects their capacity to actively metabolize and transform not only dietary fatty acids but also nonlipid nutrient components, such as proteins and carbohydrates.

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