A PRELIMINARY STUDY ON THE PURIFIED TEST DIET FOR YOUNG MILKFISH, CHANOS CHANOS*

by

Dong-Liang Lee and I-Chiu Liao**

Abstract

In studying the nutritional requirements of young milkfish, experiments were conducted to develop a purified test diet. Mixtures of the purified constituents tested were: vitamin-free casein, vitamen-free gelatin, supplemented with L-tryptophan and L-cystine as the protein sources; shark liver oil and soybean oil as the far sources; and dextrin as the carbohydrate source. Mineral mixture and vitamin mixture were also added.

The results showed that a test diet containing vitamin-free casein supplemented with L-tryptophan as the protein source, was best for the growth of young milkfish. Soybean oil was found to be a better source of fat. Vitamin mixture (4%) and mineral mixture (10%) were observed to promote growth in young milkfish. A purified test diet consisting of vitamin-free casein 60%, L-tryptophan 0.5%, soybean oil 10%, vitamin mixture 4%, mineral mixture 10%, carbohydrate and others 16% was thus suggested for young milkfish.

Introduction

Milkfish farming was well established in Taiwan for more than three hundred years and had played an important role in the island's food economy ever since. However, there is hardly any information available on the nutritional requirements of milkfish. It is fundamental to develop a purified test diet which maintains the normal growth.

The present study is largely based on the purified test diet tried on eel (Arai et al., 1971) with some modification. In this preliminary investigation, an attempt has been made to find out as ideal source of protein and fat, and the optimum quantity of vitamin mixture and

---

*Contribution A No. 33 from the Tungkang Marine Laboratory

**Mr. Lee is a Senior Specialist, while Mr. Liao is a Senior Specialist and Director, at the Tungkang Marine Laboratory, Taiwan Fisheries Research Institute, Tungkang, Pingtung, Taiwan.

mineral mixture needed for the normal growth of young milkfish. The effect of supplementary amino-acid on the growth of milkfish was also studied.

Materials and Methods

Experimental fish and feeding method

The experimental fish were young milkfish originally imported from Indonesia in October, 1974. They were overwintered in fishpond and reared in cement tank with purified diet for six weeks prior to the initiation of the present study. Fish of about 1.7 g in body weight were chosen and transferred to grey PVC aquaria (50 cm x 25 cm x 15 cm deep), each containing 25 fishes. Aeration and running water at a velocity of 1.5 liter/min were provided throughout the experiment. During the experiment period, water temperature and salinity ranged from 25 to 28°C and from 24 to 30 °/oo respectively. At 9:00 in the morning, fish were fed with the compound feed formed in the shape of noodles by pressing through 50 ml nylon syringes. The feeding was repeated every 2.5 hrs, totaling four times each. day. The aquaria were cleaned every two days and once in every two weeks the body weight of experimental fish was taken after anesthesizing in 1.0 ppm MS-222 solution.

Test diets

The main constituents of test diets are vitamin-free casein of Difco Laboratory (U.S.A.) and vitamin-free gelatin of the Matheson Company Inc. (U.S.A). Mineral mixture and vitamin mixture were added as recommended for chinook salmon Halver, (1975) except that the amount of vitamin added was doubled. In making the test diets, vitamin-free casein, dextrin, mineral mixture, vitamin mixture, amino acids and carboxymethyl cellolose were mixed in mortar first and then oil and solution of vitamin-free gelatin in water were added. The compound feed was preserved at -20°C in a freezer.

Results and Discussion

Experiment on protein

The effect of various protein on the growth of young milkfish was investigated. The composition of five test diets including various contents of vitamin-free casein and vitamin-free gelatin as protein sources for this experiment is given in Table 1. Each diet had a total of 60% protein content.

Growth of young milkfish that fed on these five test diets for 36 days is shown in Table 2 and illustrated in Fig. 1. There was best growth of fish in Lot 1 that fed on diet with only vitamin-free casein as protein source. The higher the content of vitamin-free gelatin in the test diet was, the poorer the growth obtained. The probable reason might be that gelatin was hard for young milkfish to digest or that the amino acid imbalance influenced the growth rate of milkfish in the presence of gelatin.

Experiment on fat

The effect of different sources of fat in the test diet on the growth of young milkfish was investigated. The test diet consisting of vitamin-free casein, vitamin-free gelatin, vitamin mixture, mineral mixture and dextrin was supplemented with 10% fat. Six test diets with various amount of shark liver oil and soybean oil were made (Table3.) for this experiment.

Data on the growth of young milkfish fed on these six test diets for 36 days is tabulated in Table 4 and illustrated in Fig. 2. The best growth was observed in Lot 6 of the diet with only soybean oil as fat source. Poorer growth resulted when fed with lower content of soyben oil and higher content of shark liver oil. The worst growth of fish was observed in Lot 10 fed on the test diet containing only shark liver oil as the fat source. The longer the term for feeding, the slower the growth rate was. In Lots 9 and 10 with higher content of shark liver oil, 30% of experimental fish showed injury of fins after being reared for 36 days. This may probably be due to the deficiency of essential fatty acids or poisonous effect of peroxide of shark oil liver.

The fat requirement usually differs in different species of fish. For example , Halver (1957) found that corn oil was best for chinook salmon, while compound of corn oil and fish liver oil was found best for rainbow trout and eel by Halver and Coates (1957) and Arai et al.

Table 1. Composition of the test diets in experiments with protein

Lot No. / Ingredients	1	2	3	4	5
Vitamin-free casein	56	51	46	41	36
Vitamin-free gelatin	0	5	10	15	20
Soybean oil	8	8	8	8	8
Shark liver oil	2	2	2	2	2
Vitamin mixture*	4	4	4	4	4
Mineral mixture	4	4	4	4	4
Dextrin	14.5	14.5	14.5	14.5	14.5
L Tryptophan	0.5	0.5	0.5	0.5	0.5
L-Cystine	1	1	1	1	1
Carboxymethyl cellulose	10	10	10	10	10
Water	200	200	200	200	200

*Vitamin-free casein was used as carrier for vitamin mixture and the amount of vitamin mixture added to each diet was the same as that reported by Halver (1957).

Table 2. Growth of young milkfish in experiment with protein.

Lot no.	Number of fish	Average body weight (g)		Growth rate (%)
		Initial	Final	
1	25	1.65 ± 0.64	3.09 ± 0.90	86.93
2	25	1.77 ± 0.64	2.70 ± 0.96	52.19
3	25	1.60 ± 0.45	2.53 ± 0.85	58.22
4	25	1.59 ± 0.49	2.76 ± 0.99	73.80
5	25	1.56 ± 0.43	2.36 ± 0.92	51.67

Table 3. Composition of the test diets in experiment with fat.

Lot No. / Ingredients	6	3	7	8	9	10
Vitamin-free casein	46	46	46	46	46	46
Vitamin-free gelatin	10	10	10	10	10	10
Soybean oil	10	8	6	5	2	0
Shark liver oil	0	2	4	5	8	10
Vitamin mixture*	4	4	4	4	4	4
Mineral mixture	4	4	4	4	4	4
Destrin	14.5	14.5	14.5	14.5	14.5	14.5
L-Tryptophan	0.5	0.5	0.5	0.5	0.5	0.5
L-Cystine	1	1	1	1	1	1
Carboxymethyl cellulose	10	10	10	10	10	10
Water	200	200	200	200	200	200

*Vitamin-free casein was used as carrier for vitamin mixture and the amount of vitamin mixture added to each diet was the same as that reported by Halver (1957).

Table 4. Growth of young milkfish in experiment with fat.

Lot No.	Number of fish	Average body weight (g)		Growth rate (%)
		Initial	Final	
6	25	1.74 ± 0.48	2.79 ± 0.98	59.88
3	25	1.60 ± 0.45	2.53 ± 0.85	58.22
7	25	1.59 ± 0.46	2.41 ± 0.92	51.19
8	25	1.63 ± 0.49	2.25 ± 0.86	38.37
9	25	1.38 ± 0.31	2.03 ± 0.45	46.78
10	25	1.77 ± 0.53	2.22 ± 0.68	25.42

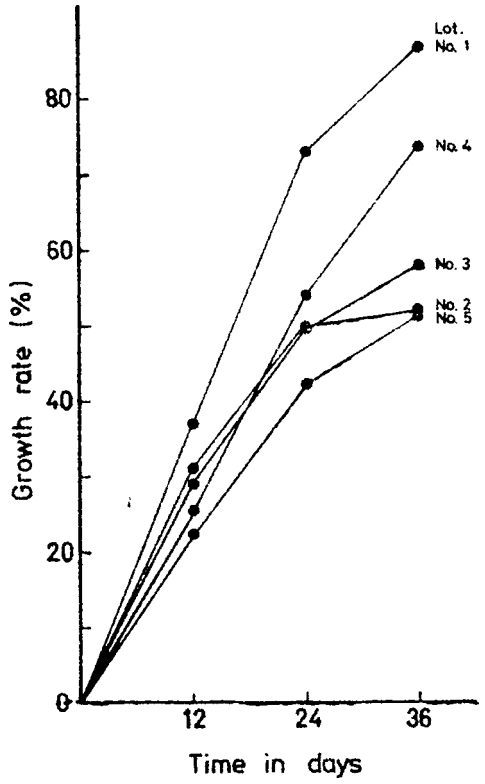

Fig. 1 Body weight gain in various lots of experiment on protein.

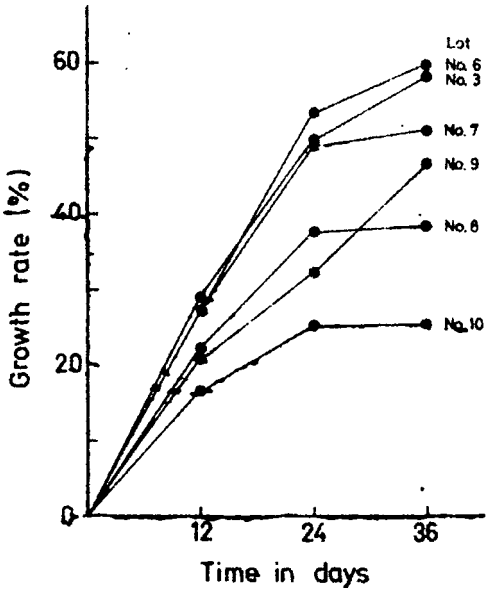

Fig. 2 Body weight gain in various lots of experiment on fat.

(1971) respectively. The growth of both rainbow trout (McLaren et al., 1947) and yellowtail (Tsukahara et al., 1967) was not good when fed with corn oil alone as the fat source but could be improved with supplement of fish liver oil, Yone et al. (1971) found that sea bream grew well with fish liver oil as its only fat source. However, the present experiment demonstrated that soybean oil was quite good for the growth of yound milkfish.

Experiment on vitamin mixture and mineral mixture

In order to study the effect of vitamin mixture and mineral mixture on the growth of young milkfish, the experiment was done by following the prescription of Halver (1957) on chinook salmon. As shown in Table 5, four test diets containing various quantity of vitamin mixture ranging from 0% to 6% and five test diets containing various quantity of mineral mixture ranging from 2% to 10% were tested.

The results obtained after rearing for 36 days showed that young milkfish grew better as the vitamin mixture content was increased up to 4% but the growth rate was lower when increased to 6%. The retardation of appetite and body weight started on the 12th day in Lot 11 which were fed on the vitamin-free test diet. It was followed by high mortality which increased to 50% on the 36th day. The result is presented in Table 6 and illustrated in Fig. 3.

As has been noted in the present experiment, young milkfish showed low appetite, decreased body weight and high mortality when vitamin in diet was insufficient. Although vitamin is essential for fish nutrition, it is generally believed that overdose of vitamin is not of any use.

The result of experiments conducted on the requirement of mineral mixture is presented in Table 6 and illustrated in Fig. 4. There was no remarkable difference in growth among young milkfish fed on the diet containing various quantity of mineral mixture. Only from Lot 17 was obtained and so it seemed that 10% mineral mixture would be beneficial for young milkfish.

It is well known (Phillips, 1959), that fish get the necessary minerals from environmental waters through gills and skin. Such capctity, however, varies from species to species. Wolf (1951) found no bad effect when rainbow trout was fed with mineral-deficient diet

Table 5. Composition of the test diets in experiment with vitamin mixture and mineral mixture.

	Experiment of vitamin mixture				Experiment of mineral mixture				
Lot No.	11	12	3	13	14	3	15	16	17
Ingredients									
Vitamin-free casein	50	48	46	44	46	46	46	46	46
Vitamin-free gelatin	10	10	10	10	10	10	10	10	10
Soybean oil	8	8	8	8	8	8	8	8	8
Shark Liver oil	2	2	2	2	2	2	2	2	2
Vitamin mixture*	0	2	4	6	4	4	4	4	4
Mineral mixture	4	4	4	4	2	4	6	8	10
Dextrin	14.5	14.5	14.5	14.5	16.5	14.5	12.5	19.5	8.5
L-Tryptophan	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
L-Cystine	1	1	1	1	1	1	1	1	1
Carboxymethyl cellulose	10	10	10	10	10	10	10	10	10
Water	200	200	200	200	200	200	200	200	200

*Vitamin-free casein was used as carrier for vitamin mixture and the amount of vitamin mixture added to each diet was the same as that reported by Halver (1957).

Table 6. Growth of young milkfish in experiment with vitamin mixture and mineral mixture.

Lot No.		Number of fish	Average body weight (g) Initial	Average body weight (g) Final	Growth rate (%)
Experiment	11	25	1.85 ± 0.62	---	---
of vitamin	12	25	1.62 ± 0.31	2.32 ± 0.57	43.56
mixture	3	25	1.60 ± 0.45	2.53 ± 0.85	58.22
	13	25	1.60 ± 0..45	2.21 ± 0.69	37.86
Experiment	14	25	1.34 ± 0.18	2.15 ± 0.48	60.32
of mineral	3	25	1.60 ± 0.45	2.53 ± 0.85	58.22
mixture	15	25	1.53 ± 0.39	2.31 ± 0.70	50.58
	16	25	1.59 ± 0.60	2.42 ± 0.94	52.01
	17	25	1.73 ± 0.56	2.92 ± 0.99	68.00

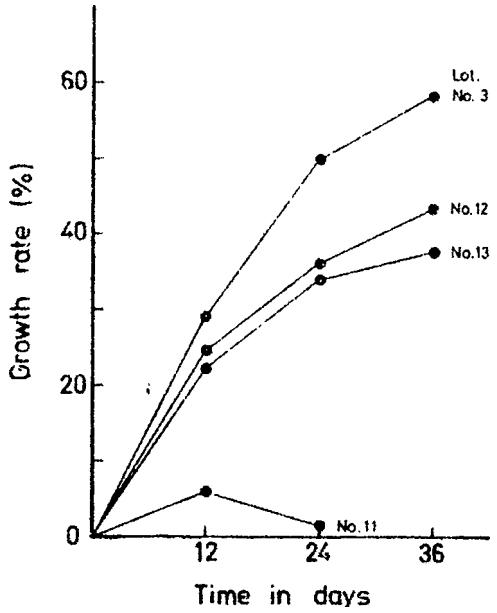

Fig. 3 Body weight gain in various lots of experiment on vitamin mixture.

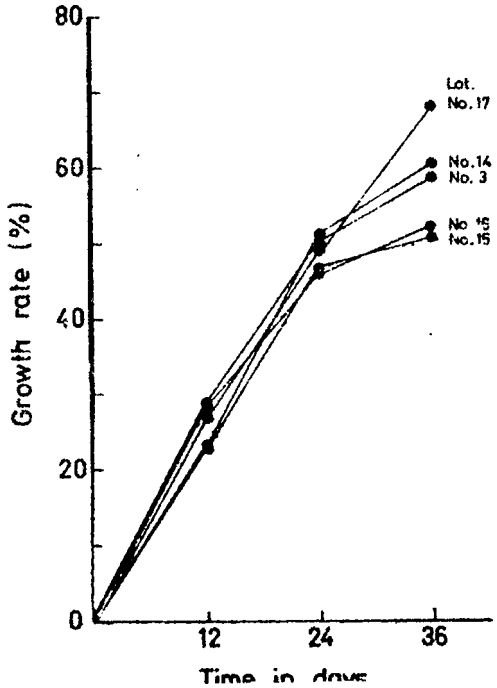

Fig. 4 Body weight gain in various lots of experiment on mineral mixture.

for a long period of 23 weeks. On the contrary, Arai et al. (1971) reported the syndrome of low appetite hindered growth and high mortality in eel fed on mineral-deficient diet in the second week. They obtained best growth in the group fed with 8% mineral mixture. Young milkfish required approximately 10% mineral mixture in diet, close to that found in eel.

Experiment on supplement of amino acids

It is noted in the experiment on protein that milkfish grew well when fed on the diet with vitamin-free casein as the only protein source. For demonstration the effect of vitamin-free casein supplemented with crystalline L-amino acid, seven test diets with various amount of tryptophan, cystine, methionine and threonine as shown in Table 7 were tested.

The experiment was conducted for 28 days and it was found that all groups with supplementary amino acid had better growth than those without it (Table 8 and Fig. 5). Especially, fish in Lot 21 with 0.5% tryptophan showed the most satisfactory growth. From these results, it is believed that the amino acid balance would be more suitable for young milkfish after the supplement of tryptophan made the tryptophan content in casein more complete. Lots 19, 20 and 23 showed a decreased effect of supplementing with 0.5% tryptophan and additional cystine, methionine and threonine that might have changed the amino acid balance. Similar result was reported by Arai et al. (1971) in their study on test diet for eel. There was significant increase of eel growth when fed on the diet containing casein and gelatin as protein sources supplemented with 0.5% tryptophan and 1% cystine. However, an additional 0.5% threonine and 1% methonine showed the bad effect on its growth.

From the results obtained in above experiments, a purified test diet consisting of vitamin-free casein 60%, L-tryptophan 0.5%, soybean oil 10%, vitamin mixture 4%, mineral mixture 10%, carbohydrate and others 16% was suggested for young milkfish.

Acknowledgements

This study was supported by the National Science Council. We gratefully thank all the members of Tungkang Marine Laboratory, we are especially indebted to N. H. Chao, C. C. Tseng, S. W. Lou and C. S. Cheng for their generous help.

Table 7. Composition of the test diets in experiment with supplement of amino acids.

Ingredient / Lot No.	18	19	20	21	22	23	24
Vitamin-free casein	51.5	50	50	51	50.5	49.5	49.5
Soybean oil	10	10	10	10	10	10	10
Vitamin mixture*	4	4	4	4	4	4	4
Mineral mixture	10	10	10	10	10	10	10
Dextrin	14.5	14.5	14.5	14.5	14.5	14.5	14.5
L-Tryptophan		0.5	0.5	0.5		0.5	
L-Cystine		1				1	1
L-Methionine			1		1		1
L-Threonine						0.5	
Carboxymethyl cellulose	10	10	10	10	10	10	10
Water	200	200	200	200	200	200	200

*Vitamin-free casein was used as carrier for vitamin mixture and the amount of vitamin mixture added to each diet was the same as that reported by Halver (1957).

Table 8. Growth of young milkfish in experiment with supplement of amino acids.

Lot No.	Number of fish	Average body weight (g) Initial	Average body weight (g) Final	Growth rate (%)
18	20	4.06 ± 1.45	5.02 ± 1.69	23.63
19	20	4.14 ± 1.34	5.16 ± 1.61	24.70
20	20	3.95 ± 1.29	5.18 ± 1.88	31.06
21	20	3.77 ± 1.33	5.06 ± 2.15	33.99
22	20	3.89 ± 1.10	4.94 ± 1.80	26.79
23	20	3.82 ± 1.25	4.95 ± 1.68	29.58
24	20	4.12 ± 1.27	5.28 ± 1.82	28.05

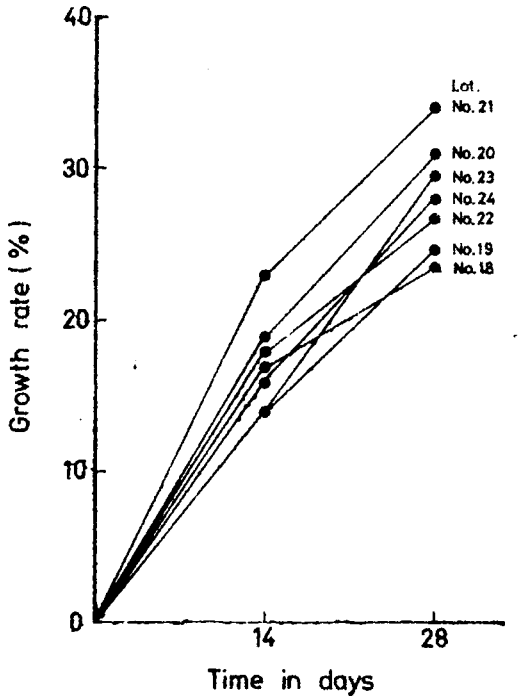

Fig. 5 Body weight gain in various lots of experiment on supplement of amino acids.

References

Arai S., T. Nose and Y. Hashimoto. 1971. A purified test diet for the eel, Anguilla japonica. Bull. Freshwater Fish. Res. Lab., 21: 161-178

Halver, J. E. 1957. Nutrition of salmonoid fishes III. Water-soluble vitamin requirements of chinook salmon. J. Nutrition, 62: 225-243

Halver, J. E. and J. A. Coates. 1957. A vitamin test diet for long-term feeding studies. Prog. Fish-Cult., 19 : 112-118

McLaren, B. A., E. Keller, D. J. O'Donnell and C. A. Elvehjem. 1947. The nutrition of rainbow trout-- I. Studies of vitamin requirements. Arch. Biochem., 15 : 169-178

Phillips, A. M. Jr. 1959. The known and possible role of minerals in trout nutrition and physiology. Trans. Amer. Fish. Soc. 88 : 133-135

Tsukahara, H., A. Furukawa and K. Funae. 1967. Studies on feed for fish VII. The effects of dietary fat on the growth of yellowtail. Bull. Naikai Regional Fish. Res. Lab., 24 : 29-50 (in Japanese with English abstract)

Wolf, L. E. 1951. Diet experiments with trout. Prog. Fish-Cult., 13: 17-24.

Yone, Y., M. Furuichi and S. Sakamoto. 1971. Studies on nutrition of red sea bream--III. Nutritive value and optimum content of lipids in diet. Report of Fish. Res. Lab., Kyushu University, 1 : 49-60 (in Japanese with English abstract)