Bull. Inst. Zool., Academia Sinica 16(2): 91-98 (1977)

NUTRITIVE VALUES OF LEAF PROTEIN CONCENTRATES FOR GRASS CARP *CTENOPHORYNGODON IDELLUS*[1,2]

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Received for publication, Nov. 20, 1977

ABSTRACT

Lu Jan, Ya-Li Hsu and Jen-Leih Wu (1977) *Nutritive Values of Leaf Protein Concentrates for Grass Carp Ctenophoryngodon idellus.* Bull. Inst. Zool., Academia Sinica, 16(2): 91-98. After five weeks feeding with 20% leaf protein concentrates, the nutritive values of those proteins were assessed by body weight change, "Net Protein Ratio" (NPR) and "Net Protein Utilization" (NPU). Among the leaf protein concentrates tested, the protein from Chinese milk vetch showed best protein quality for grass carp (*Ctenophoryngodon idellus*). Other proteins had the quality in the following decreasing sequence: napier, sugar cane top, sun hemp, miscanthus, and tienching. Some leaf protein concentrates such as tienching and miscanthus caused serious mortality. The only protein without lethal effect on grass carp was Chinese milk vetch. The protein quality of Chinese milk vetch could be improved significantly by casein or by essential amino acid supplementation.

The feeding habit of grass carp (*Ctenophoryngodon idellus*) is herbivorous[12]. Thus the traditional grass carp culture is fed by herbaceous leaves. Usually the fresh grass leaves have very low protein content (1 to 4%)[20], therefore, the grass carp needs large amount of grass for growth. As communicated to the authors by fish farmers, the grass carp may consume daily fresh grass mass as much as 50% of its body weight, and sufficient supply of fresh grass becomes a limiting factor for single culture of grass carp. Also, the feces from grass carp contains considerable amount of undigested plant cells (unpublished data). In order to improve the dietary efficiency of grass protein for grass carp culture, two important factors should be explored; improvement of nutritional quality of grass protein and increase of protein concentration in the fish diet. However, very limited work has been done on the protein nutrition of grass carp[5,17].

Recently the leaf protein concentrates have gained attention as a cheap protein source for human and animals[10,16]. The nutritive value of leaf protein concentrates have been evaluated in domestic animals[1,15]. However, the nutritive value of these proteins for grass carp is still not known. In this study, the protein quality of leaf protein concentrates from Chinese milk

1. Paper No. 190 of the Journal Series of the Institute of Zoology, Academia Sinica.
2. This research was supported by Grant No. NSC-66B-0201-03 (26) from the National Science Council, Republic of China

vetch, miscanthus, napier, sugar cane top, sun hemp and tienching for grass carp was evaluated by biological methods, e. g. the evaluation by body weight increase, "Net Protein Ratio" and "Net Protein Utilization". The quality of Chinese milk vetch leaf protein concentrate could be improved by casein or free amino acid supplementation. The toxic effects of some leaf protein concentrates were also revealed.

MATERIALS AND METHODS

I. Fish

The fingerlings of *Ctenophoryngodon idellus* with a body weight of about 3 gm were bought from the rearing pond near Lukang, Taiwan. The fish were fed with a soybean diet before the feeding test.

II. Leaf Protein Concentrates

Leaf protein concentrate of Chinese milk vetch (*Astragalus sinicus*) was obtained from Dr. T. Y. Liu, Food Industry Research and Development Institute. Miscanthus leaf protein concentrate (*Miscanthus floridulus*) prepared by acid precipitation method[6] was supplied by Dr. C.H. Chou, Institute of Botany, Academia Sinica. Leaf protein concentrates from napier (*Pennisetum purpureum*), sugar cane top (*Saccharum officinarum*), sun hemp (*Crotalaria juncea*) and tienching (*Sesbania sesban*) were supplied by Mr. C. Y. Peng, Taiwan Sugar Research Institute.

III. Feeding Method

One week before the experiment, 15 fingerlings each were transferred to an aquarium and fed on different test diet for adaptation. The daily feeding amount of the dried diet was 2% of the total body weight of individual group. The amount was adjusted according to the total body weight change every week. The fish were fed three times a day at 8 a. m., 11 a. m. and 3 p. m.. The feeding on test diets was lasted for 5 weeks. The water temperature was maintained at 27°C throughout the experiment. The aquarium was renewed with dechlorinated and well-aerated tap water every two days.

TABLE 1
The composition of different leaf protein concentrate diets.

Ingredient \ Diet No.	1	2	3	4	5	6	7	8
Casein	24.1	—	—	—	—	—	—	—
Chinese milk vetch	—	37.7	—	—	—	—	—	—
Miscanthus	—	—	68.3	—	—	—	—	—
Napier grass	—	—	—	40.9	—	—	—	—
Sugar cane top	—	—	—	—	69.2	—	—	—
Sun hemp	—	—	—	—	—	32.8	—	—
Tienching	—	—	—	—	—	—	36.6	—
Dextrin	45.9	32.3	1.7	29.1	0.8	37.2	33.4	70
α-Starch	15	15	15	15	15	15	15	15
Cellulose powder	5	5	5	5	5	5	5	5
Soybean oil	3	3	3	3	3	3	3	3
Cod liver oil	2	2	2	2	2	2	2	2
Mineral premix	4	4	4	4	4	4	4	4
Vitamin premix	1	1	1	1	1	1	1	1
Protein level (%)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	0

IV. Test Diets

The compositions of test diets were listed in Tables 1, 2 and 3. The compositions of mineral premix and vitamin premix were the same as those described in the previous report[19]. The total crude protein level in Table 1 was 20%. L-forms of the essential amino acids (histidine, lysine, methionine and valine) were supplemented to the protein concentrate of Chinese milk vetch to the same amino acid content as that in 30% casein diet (Table 3). The pelleted ration was made by mixing the dry components with hot water at 80°, pressing through a meat mincer, and cutting into crumbles of the size of about 1.5 × 5.0 mm. After air-dried for 2 to 3 hours at room temperature, the pellets were stored in a freezer at −20°.

V. Data Analysis

The body weight of each fish was determined at the beginning of the experiment and weekly thereafter. At the end of the feeding experiment, the final body weight was recorded and the carcass was dried at 105°, overnight, followed by grinding to a homogeneous powder. The total nitrogen of each sample and leaf protein concentrates was determined by micro-Kjeldahl method[18]. The crude protein was calculated by total nitrogen × 6.25.

TABLE 2
The composition of different protein level of Chinese milk vetch diet.

Ingredient \ Diet No.	1	2	3
Chinese milk vetch	56.53	37.69	18.84
Dextrin	13.47	32.31	51.16
α-Starch	15	15	15
Cellulose powder	5	5	5
Soybean oil	3	3	3
Cod liver oil	2	2	2
Mineral premix	4	4	4
Vitamin premix	1	1	1
Protein level (%)	30.0	20.0	10.0

TABLE 3
The composition of diets prepared by supplementing casein or essential amino acids to Chinese milk vetch protein concentrate.

Ingredient \ Diet No.	1	2	3	4	5
Casein	36.14	—	24.10	12.05	12.05
Chinese milk vetch	—	56.53	18.84	37.69	—
Histidine	—	0.21	—	—	—
Lysine	—	0.51	—	—	—
Methionine	—	0.39	—	—	—
Valine	—	0.21	—	—	—
Dextrin	33.86	13.47	27.06	20.26	57.95
α-Starch	15	15	15	15	15
Cellulose powder	5	5	5	5	5
Soybean oil	3	3	3	3	3
Cod liver oil	2	2	2	2	2
Mineral premix	4	4	4	4	4
Vitamin premix	1	1	1	1	1
Protein level (%)	30.0	30.0	30.0	30.0	30.0

The nutritive values of leaf protein concentrates were expressed as "Net Protein Ratio" (NPR) and "Net Protein Utilization" (NPU) which calculated by the following formula[2]:

$$NPR = \frac{\text{Final body weight of the fish receiving a test protein diet} - \text{Final body weight of the fish receiving a non-protein diet}}{\text{Amount of protein intake of test group}}$$

$$NPU = \frac{\text{Final carcass protein of the fish receiving a test protein diet} - \text{Final carcass protein of the fish receiving a non-protein diet}}{\text{Amount of protein instake of test group}} \times 100$$

RESULTS AND DISCUSSION

I. Nutritive values of leaf protein concentrates

In order to understand the quality of the protein concentrates from Chinese milk vetch, miscanthus, napier, sugar cane top, sun hemp and tienching for grass carp (*Ctenophoryngodon idellus*), the diets containing 20% of the crude protein of the above protein sources were fed to grass carp for 5 weeks. The pictures comparing the appearance of the typical fish selected from different protein diets groups with fish from the control group are shown in Fig. 1. It is clearly shown the protein quality can affect the nutritional state of grass carp. The average body weight was measured every week and shown in Fig. 2. The grass carp could continuously increase its body weight on casein diet during the experimental period. The final body weight was 17.45% over the initial body weight. However, the leaf protein concentrates tested in this study showed decreased average body weight from the beginning except that Chinese milk vetch could increase the fish body weight during the first two weeks. After two weeks feeding, the body weight of the group on Chinese milk vetch diet started to decline and the final body weight was only 97.65% of the beginning. (Fig. 2). After five weeks feeding on non-protein diet, the final body weight was 79.19% of the beginning. The body weight loss of the groups on sugar cane top, napier grass and sun hemp diets was not so serious as that on non-protein diet. On the miscanthus diet, the final body weight loss was almost the same as the non-protein diet, but the tienching diet showed much more serious body weight loss compared with that of non-protein diet. The final body weight of tienching diet was only 72.82% of the beginning.

Based on the body weight difference among the test groups fed with leaf protein concentrates and protein-free diet, the nutritive value of leaf protein concentrates were expressed as "Net Protein Ratio" (NPR) and shown in Table 4. This calculation indicated that the ingested

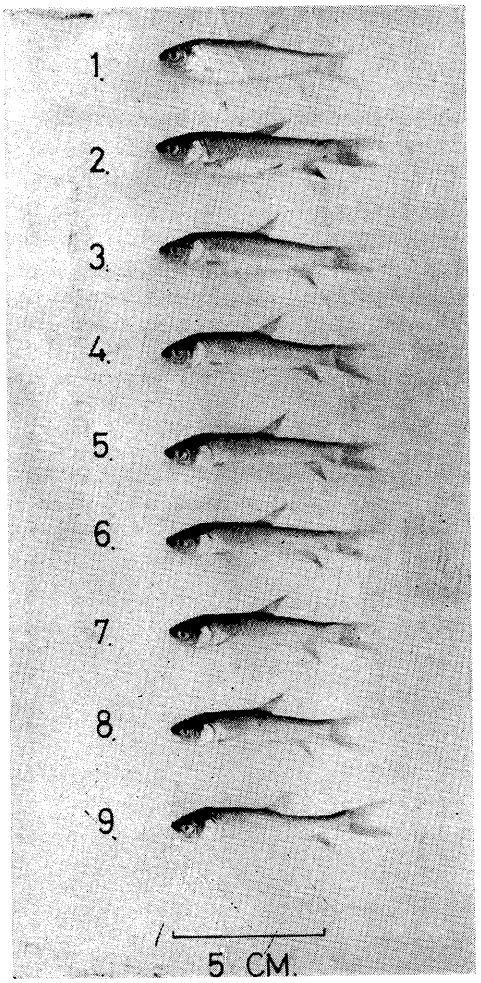

Fig. 1. The appearance of grass carp fed on different protein diets.
1. soybean; 2. casein; 3. Chinese milk vetch; 4. sugar cane top; 5. sun hemp; 6. napier; 7. miscanthus; 8. non-protein; 9. tienching.

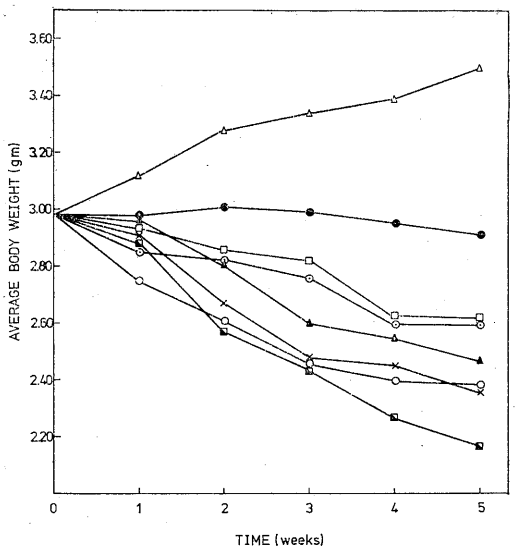

Fig. 2. The body weight change of grass carp fed on different protein diets.
△: casein; ●: Chinese milk vetch;
□: sugar cane top; ⊙: napier;
▲: sun hemp; ○: miscanthus;
×: non-protein; ◪: tienching.

proteins were utilized for maintenance and growth. Among the leaf protein concentrates tested, the Chinese milk vetch had the highest NPR value which was 1.54. The NPR values of other leaf proteins were rather low compared with that of casein diet and had the following decreasing sequence: napier grass, sugar cane top, sun hemp, miscanthus and tienching. The tienching protein could not be utilized for maintenance as indicated by a negative NPR value. The index of nutritive value of leaf protein concentrates based on change in body nitrogen content, the "Net Protein Utilization" (NPU), was calculated and shown in Table 4. Chinese milk vetch had NPU value of 33.15, which was the highest among the leaf proteins. The nitrogen retained in the fish on other leaf protein diets was low. The NPU values of napier grass, sun hemp, sugar cane top, miscanthus and tienching were 12.83, 11.17, 9.58, 5.84 and −50.62, respectively. From these two nutritional indices, it is seen that the leaf protein concentrate from Chinese milk vetch has the best quality for grass carp feeding.

By comparing the essential amino acid patterns, Chinese milk vetch has lower histidine, lysine, methionine and valine contents than casein[2,3], and miscanthus has lower lysine and methionine contents than casein[6]. Although miscanthus leaf protein has a better essential amino acid pattern than Chinese milk vetch, the growth condition on miscanthus diet was poorer than on Chinese milk vetch. Thus the

TABIE 4

Body weight change, "Net Protein Ratio" and "Net Protein Utilization" of different protein diets fed to *Ctenophoryngodon idellus*.

Protein Source	Average Body wt. (gm)		Protein intake (mg)	NPR	Carcass protein at the end (mg)	B_f-B_k*(mg)	NPU(%)
	Initial	Final					
Casein	2.98	3.50	406	2.81	430.15	199.75	49.20
Chinese milk vetch	2.98	2.91	358	1.54	349.06	118.66	33.15
Miscanthus	2.98	2.38	236	0.08	244.19	13.79	5.84
Napier grass	2.98	2.61	324	0.77	271.96	41.56	12.83
Sugar cane top	2.98	2.62	364	0.71	265.27	34.87	9.58
Sun hemp	2.98	2.47	278	0.40	261.45	31.05	11.17
Tienching	2.98	2.17	84	−2.26	187.88	− 42.52	−50.62
Non-protein	2.98	2.36	—	—	230.40	—	—

* B_f: Final carcass protein of the fish receiving a test protein diet.
B_k: Final carcass protein of the fish receiving a non-protein diet.

essential amino acid content is not the only factor to be considered in grass carp culture. Other factors such as the presence of non-protein nitrogen compounds which reduce the actual protein level in the diet[8]; the presence of toxic factor or protease inhibitor[14] to hinder growth and cause death (discussed later); and the different digestibility among leaf proteins[11], should also be taken into account.

The varied level of mortality was observed in the different protein diets groups (Fig. 3). The control group (non-protein diet) started to show death after 3 weeks' and the final mortality was 13.3%. However, the tienching diet-induced death occurred after one week feeding. Most of the fish died between the 3rd and 4th weeks. The final mortality of the tienching diet group was 93.3%. The miscanthus and sun hemp could cause fish death after two weeks' feeding and the final mortality were 53.3% and 33.3%, respectively. Napier grass group had a lethal pattern similar to that on non-protein diet. Although the sugar cane top initiated fish death as late as at the 5th week, the final mortality was 20%, which was higher than that caused by non-protein diet. The only groups which did not cause death were casein diet and Chinese milk vetch diet. The cause of serious fish death was not clear. The possible explanations could be: (i) saponins or other toxic substances are present in the test diets[4]; (ii) toxic non-protein amino acids may be present[9], (iii) protease inhibitors hinder the protein digestion[14,18], (iv) some toxic substances were introduced during the preparation of leaf protein concentrates. The toxic factor(s) must be identified before using the leaf protein concentrate as the protein source in grass carp culture.

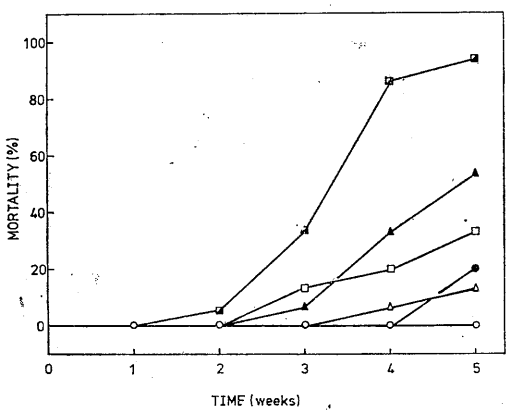

Fig. 3. Mortality of grass carp fed on different protein diets:
◪: tienching; ▲: miscanthus;
□: sun hemp; ●: sugar cane top;
△: napier or non-protein;
○: casein or Chinese milk vetch.

II. Supplementation of Chinese milk vetch diet.

Different protein levels of Chinese milk vetch diet were fed to grass carp for 5 weeks. At 20% protein level, the average body weight had a slight decrease, but at 10% protein level, serious body weight loss ensued (Fig. 4). By increasing the Chinese milk vetch protein to 30%, the average body weight increased only 2.14% over the initial body weight. Therefore, the Chinese milk vetch protein has a limited nutritive value for grass carp growth. In order to understand the supplementation effect of Chinese milk vetch protein, casein or essential amino acids were added (Fig. 5). By adding 10% casein protein to 20% Chinese milk vetch protein diet, the growth rate increased and the final body weight increased by 7.12%. By

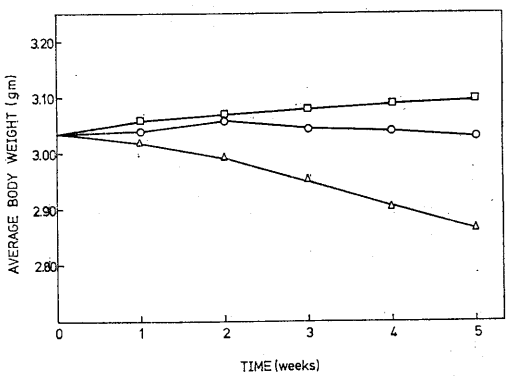

Fig. 4. The body weight change of grass carp fed on different levels of Chiness milk vetch protein.
□: 30%; ○: 20%; △: 10%.

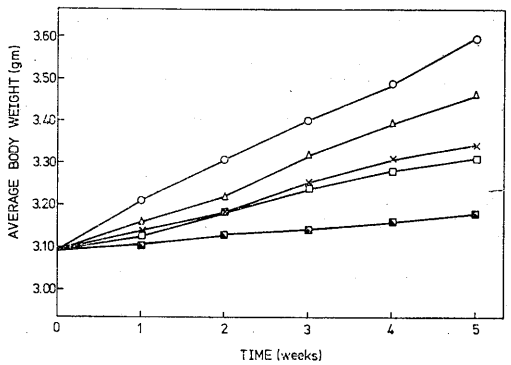

Fig. 5. The body weight change of grass carp fed on Chinese milk vetch with casein or essential amino acid supplementation.
○: 30% casein;
△: 10% Chinese milk vetch+20% casein;
□: 20% Chinese milk vetch+10% casein;
◪: 10% casein;
×: 30% Chinese milk vetch+0.21% his+0.51% lys+0.39% met+0.2% val.

comparing the growth of grass carp on 10% casein diet or on 20% Chinese milk vetch diet with that on 10% casein plus 20% Chinese milk vetch, it is noted that the nutritive value of Chinese milk vetch can be improved by casein protein. The fish on 20% casein plus 10% Chinese milk vetch protein diet had a much higher growth rate (12.46%) than on the individual protein alone. Essential amino acids were supplemented to Chinese milk vetch diet to levels as high as those in casein. As shown in Fig. 5, supplementation of the amino acids had 8.19% increase over the initial body weight. This result indicates that the addition of casein protein or essential amino acids can improve the nutritive value of Chinese milk vetch by balancing the amino acid pattern. However, suplementation of essential amino acid in free form resulted in the increase of body weight only 50% of that on 30% casein diet. This may be due to a very delicate amino acid balance in casein protein for grass carp[7]. Also, the utilization of free form of amino acids is somewhat different from the polypeptide form. Further investigation on the balanced amino acid diet for grass carp is worthwhile.

Acknowledgement: We thank Dr. Jong-ching Su for his helpful suggestions and his reading of this manuscript. We also thank Dr. T. Y. Liu, Dr. C. H. Chou and Mr. C. Y. Peng for generous supply of various leaf protein concentrates used in this experiment.

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葉蛋白濃縮物對於草魚 *Ctenophoryngodon idellus* 之營養價值

詹璐　徐亞莉　吳金洌

爲了探討葉蛋白濃縮物對於草魚 *Ctenophoryngodon idellus* 之營養價值，將幼草魚分別飼以 20%蛋白含量之不同葉蛋白濃縮物，五週之後，測定其體重變化，淨蛋白質比率及淨蛋白質利用價等營養指標。結果顯示紫雲英對草魚而言，最具營養價值，其餘之葉蛋白質之營養價依狼尾草，蔗梢，太陽蔬，五節芒及田菁之順序減低，但是田菁及五節芒會引起草魚之嚴重死亡，祇有紫雲英不會引起死亡現象。紫雲英葉蛋白之蛋白質品質可以用酪蛋白或必需胺基酸之添加而改進。