ティラピアにおける数種のタンパク質の栄養価と成長に及ぼす要因について

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Effect of Several Protein Sources and Other Factors on the Growth of *Tilapia nilotica*[*1]

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The feeding trials were conducted to examine the effects of several protein sources and other factors on the weight gain, feed conversion efficiency (FCE), and protein efficiency ratio (PER) of *Tilapia nilotica*. As a protein source, casein-gelatin (3: 1) and casein had a higher nutritive value than gelatin, albumin, and a mixture of casein and crystalline amino acids (1: 1).

The effects of feeding rate, feeding frequency, stocking density, dietary vitamin level, and a binder on growth of *Tilapia* were tested with 2 levels in a factorial design using an orthogonal array L_8. As for the weight gain, FCE, and PER, a significant difference ($P<0.05$) was detected only on the feeding rate. The weight gain was higher at 7.0% feeding rate than at 3.5% feeding rate; whereas the FCE and PER were higher at 3.5% feeding rate than at 7.0% feeding rate. The weight gain, FCE, and PER of *Tilapia* were not affected by the feeding frequency, stocking density, dietary vitamin level, and the binder under the experimental conditions adopted.

Several groups of workers have studied the requirements of *Tilapia* for proteins,[1–7] lipids,[8–10] and digestible energy levels[11] using purified or semi-purified diets. In the previous study,[7,12] we have found that the optimum protein level for the growth of *Tilapia nilotica* was 30–40% when casein was used as a protein source. However, the optimum protein level in diets varies with the kind of protein sources used. Although *T. nilotica* has been shown to require 10 essential amino acids (EAA) as other fishes do,[13] the quantitative EAA requirements have not yet been manifested. Therefore, we intended to examine the nutritive value of several protein sources and supplemental crystalline amino acids.

Although the knowledge on the nutritional requirements of *T. nilotica* has been gradually accumulated, only little information[*3] is available for the effects of rearing and feeding conditions on the growth of this fish. Hence, the present study was also aimed to clarify the effects of several factors such as feeding rate, feeding frequency, *etc.* on the growth of *T. nilotica*.

Materials and Methods

The fingerlings of *T. nilotica* were obtained from a commercial *Tilapia* farm in Kagoshima, Fuji-Enterprise Co., and maintained on a commercial carp ration until used. Experiment I dealt with the effects of dietary proteins and supplemental amino acids on the weight gain, feed conversion efficiency (FCE), and protein efficiency ratio (PER). In Experiment I, the feeding trial was designed as an one-way layout experiment with 2 repetitions. The feeding trial was conducted using 8 diets (Table 2), which were almost isonitrogenous but contained different proteins (diets 1, 3, 4, 6, and 8) and casein with supplemental amino acids (diets 2, 5, and 7), under the rearing conditions given in Table 1. In the case of *Tilapia zillii*,[1] the addition of supplemental L-tryptophan and L-methionine to a casein diet seemed to improve growth slightly. Accordingly, both amino acids were supplemented to a casein (diet 2) in order to check the growth-promoting effect for *T. nilotica*. In diet 7, the half of casein was substituted with a mixture of crystalline amino acids to examine the utilization of free amino acids by this fish. Also, since casein was low in arginine and lysine in comparison with the whole body proteins of *T. nilotica* (Table 6), crystalline L-arginine HCl and L-lysine HCl were supplemented to casein in diet 5.

In Experiment II, the feeding trials were conducted to examine the effects of feeding rate (factor A), feeding frequency (factor B), stocking density (factor C), dietary vitamin level (factor D), kind of

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Table 1. Rearing conditions of *Tilapia* in Experiment I

Condition	Remark
Feeding period	4 weeks (Sept. 1 to 28)
Average initial body wt.	1.03 g
Number of fish/tank (30 *l*)	15
Feeding rate (% of body wt.)	7.0%
Daily feeding frequency	Twice (9 a.m. and 3 p.m.)
Water temperature	28°C

Table 2. Composition of test diets used in experiment I

Diet*1	Dietary protein and amino acid
1	Casein (35.0%)
2	Casein (35.0%)+L-Trp (0.5%) +L-Met (0.5%)
3	Gelatin (35.0%)
4	Albumin (35.0%)
5	Casein (35.0%)+L-Lys (2.04%) +Arg (1.57%)*2
6	Casein (38.6%)
7	Casein-Crystalline amino acids (1: 1)*3 (35.0%)
8	Casein-gelatin (3: 1) (35.0%)

*1 The basal ration of test diets was similar to that described previously and contained the following ingredients (%): Proteins and amino acids, 35.0–38.6, dextrin, 30.0; pollack liver oil, 7; soybean oil, 7; linoleic acid, 1.0; mineral mixture, 4.0; vitamin mixture, 1.0; agar, 3.0; α-cellulose (equal to 100).

*2 Crystalline L-lysine HCl and L-arginine HCl were supplemented to casein diet (diet 1) to the same levels of these amino acids as in the whole body proteins of *T. nilotica*.

*3 The half of casein in diet 1 was substituted with a mixture of crystalline amino acids.

Table 3. Factors and levels examined in Experiment II

Factor		Level	
A:	Feeding rate (% of body wt./day)	A_1:	7.0%
		A_2:	3.5%
B:	Feeding frequency*	B_1:	Twice/day
		B_2:	3 times/day
C:	Stocking density (Number of fish/tank)	C_1:	30
		C_2:	15
D:	Vitamin level	D_1:	Halver's level
		D_2:	Halver's level×2
E:	Kind of binder	E_1:	Agar
		E_2:	CMC

* Feeding time: Twice (9 a.m. and 3 p.m.) or 3 times (9 a.m., noon, and 3 p. m.).

a binder (factor E), and the interaction between factors A and B (factor A×B) on the weight gain, FCE, and PER. Experimental groups were designed using an orthogonal array L_8[14]. Table 3 shows the factors and levels examined. Table 4 indicates the allotment of these factors to the orthogonal array. The *Tilapia* fingerlings were reared for 4 weeks under the conditions listed in Tables 3 and 4. The base ration of test diets 9 to 12 was similar to that of diet 8 in Experiment I.

Weight gain, FCE, and PER data were analyzed by analysis of variance to clarify the effects of treatments and factors. Amino acid compositions were determined with a Shimadzu High Performance Liquid Chromatography (HPLC) system on a column of cation exchange resion ISC-07/S1504 (15 cm×4.0 mm i.d.). Sample proteins were hydrolyzed with 4 N methane sulfonic acid

Table 4. Experimental groups and allotment of the factors and levels to orthogonal array L_8 in Experiment II

Exptl. group	Rearing conditions*1					
	Feeding rate	Feeding frequency	Stocking density	Vitamin level	Binder	Diet given*2
1	3.5%	Twice	30 fish	1%	Agar	Diet 9
2	3.5%	Twice	15 fish	2%	CMC	Diet 12
3	3.5%	3 times	30 fish	1%	CMC	Diet 10
4	3.5%	3 times	15 fish	2%	Agar	Diet 11
5	7.0%	Twice	30 fish	2%	Agar	Diet 11
6	7.0%	Twice	15 fish	1%	CMC	Diet 10
7	7.0%	3 times	30 fish	2%	CMC	Diet 12
8	7.0%	3 times	15 fish	1%	Agar	Diet 9

*1 The factors (see Table 5) were alloted to the orthogonal array as follows: factors A (array No. 1), B (array No. 2), A×B (array No. 3), C (array No. 4), D (array No. 5), and E (array No. 6).

*2 Diets 9, 10, 11, and 12 differ from each other in the compositions of vitamins and binder and contained the same level of other ingredients (%); casein-gelatin (3: 1), 35.0; dextrin, 30.0; pollack liver oil-soybean oil (1: 1), 14.0; linoleic acid, 1.0; minerals, 4.0; L-tryptophan, 0.5; L-methionine, 0.5; α-cellulose (equal to 100%). The diets contained the following levels of vitamins and binder: diets 9 (1% vitamins, 3% agar), 10 (1% vitamins, 5% CMC), 11 (2% vitamins, 3% agar), and 12 (2% vitamins, 5% CMC).

Table 5. Results of the feeding trial in Experiment I

Diet*1	Weight gain	FCE*2	PER*3
1	195[b]	0.75[bc]	2.13[bc]
2	230[b]	0.72[bc]	2.06[bc]
3	9	0.05	0.15
4	135[a]	0.49[a]	1.41[a]
5	220[b]	0.85[c]	2.27[bc]
6	206[b]	0.73[bc]	1.94[abc]
7	130[a]	0.58[ab]	1.65[ab]
8	240[b]	0.89[c]	2.55[c]

*1 Feeding experiment with each diet was conducted in the duplicate tanks. The weight gain, FEC, and PER data are the means. The confidence limits of means of weight gain, FCE, and PER were 35.0, 0.16, and 0.46, respectively. Values within a column followed by the same letter are not significantly different ($P > 0.05$).

*2 Gain (g)/feed (g).

*3 Gain (g)/protein intake (g)

containing 0.5% tryptamine. The details of the method for amino acid analysis were described previously.[15]

Results and Discussion

Nutritive Value of Several Proteins and Supplemental Amino Acids

In Experiment I, *Tilapia* were reared with 8 diets containing different proteins and supplemental amino acids for 4 weeks. Tables 5 and 6 show the results of feeding trial and the EAA pattern of dietary proteins, respectively. As for the weight gain, diet 8 containing casein-gelatin (3: 1) apparently achieved the highest value, and diets 2, 5, 6, and 1 followed in a decreasing order. Generally, it is likely that proteins having an EAA pattern similar to those of the whole body and egg of a fish have a high nutritive value for fish.[16-19] Since casein was low in arginine than the whole body proteins of *T. nilotica* (Table 6), the higher level of arginine in casein-gelatin (3: 1) may explain partly the improved weight gain in diet 8 in comparison with a casein diet (diet 1). The weight gain of *Tilapia* seemed to improve slightly by the supplementation of EAA such as tryptophan plus methionine (diet 2) and lysine plus arginine (diet 5). However, a significant difference ($P<0.05$) was not detected between every pair of diets 1, 2, 5, 6, and 8, indicating the necessity of further experiments to withdraw the reliable conclusion on the growth-promoting effects of these supplemental amino acids. Wu and Jan have shown that growth of *Tilapia aurea* was promoted by the single supplementation of valine, phenylalanine, or isoleucine to a soybean-meal diet.[20] However, their data were not analysed statistically, and the growth-enhancing effects of these amino acids seemed to be too small to withdraw the final conclusion.

The weight gain of *Tilapia* was significantly ($P<0.05$) lower on the diets containing gelatin (diet 3), albumin (diet 4), and a 1 to 1 mixture of casein and crystalline amino acids (diet 7). Mazid *et al.*[13] have also reported that *T. zillii* grew poorly on an amino acid diet. The carp *Cyprinus carpio* and channel catfish *Ictalurus punctatus* also have been shown to utilize scarcely an amino acid diet for growth.

The FCE and PER were also high in the group fed diet 8 containing gelatin (3: 1) and diet 5 containing casein along with supplemental lysine HCl and arginine HCl, however a significant difference ($P<0.05$) was not detected between every pair of diets 1, 2, 5, 6, and 8. The supplementation of tryptophan and methionine improved neither FCE nor PER.

Considered the weight gain, FCE, and PER data together, the present study leads to the following conclusion. First, the nutritive values of gelatin,

Table 6. Essential amino acid composition (g/100 g protein) of protein sources and the whole body protein of *T. nilotica*

Amino acid	Casein	Gelatin	Albumin	Casein-gelatin (3: 1)	*Tilapia*
Thr	5.60 (1.82)*	2.32 (2.61)	3.69 (0.45)	4.77 (1.89)	5.10 (1.48)
Val	6.06 (1.97)	3.00 (3.37)	6.34 (0.76)	5.31 (2.10)	6.05 (1.75)
Met	3.08 (1.00)	0.89 (1.00)	8.29 (1.00)	2.53 (1.00)	3.45 (1.00)
Ile	5.03 (1.63)	2.01 (2.26)	5.24 (0.63)	4.29 (1.69)	5.57 (1.61)
Leu	9.38 (3.04)	4.06 (4.56)	8.51 (1.03)	8.04 (3.18)	9.77 (2.83)
Phe	5.38 (1.75)	2.45 (2.75)	3.94 (0.48)	4.64 (1.83)	3.45 (1.00)
Lys	7.54 (2.45)	5.12 (5.75)	7.54 (0.92)	6.93 (2.74)	4.36 (1.26)
His	3.20 (1.04)	1.61 (1.81)	2.39 (0.29)	2.80 (1.11)	4.00 (1.16)
Arg	4.00 (1.30)	12.19 (13.70)	4.00 (0.48)	6.04 (2.39)	7.94 (2.30)
Trp	1.95 (0.63)	0.37 (0.42)	1.95 (0.23)	1.55 (0.61)	1.10 (0.32)

* Letters in parentheses indicate the ratio (relative to methionine).

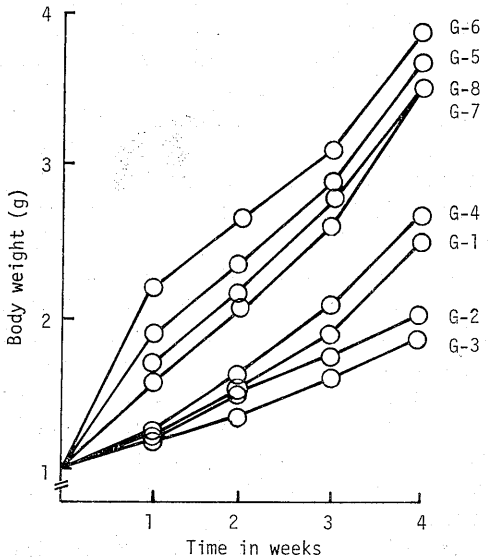

Fig. 1. Growth of *Tilapia* in Experiment II. G-1 to G-8 show the experiment groups.

albumin, the 1:1 mixture of casein and crystalline amino acids were inferior to those of casein and casein-gelatin (3:1). Second, the nutritive value of casein is possibly improved by the supplementation of arginine and lysine, the first and second limiting amino acids in casein as compared with the EAA pattern of the whole body proteins of *T. nilotica*. Although there have been several papers[23-26] showing that some *Tilapia* species grew well on diets with vegetable proteins such as soybean meal, *etc.*, animal proteins may have a higher nutritive value for *T. nilotica* than vegetable proteins.[27,28]

Table 7. Results of the feeding trial in Experiment II

Exptl. group	Mean body weight (g)		Weight gain	FCE	PER
	Initial	Final	(%)		
1	0.99	0.56	159	1.42	3.92
2	0.99	2.05	107	1.04	2.87
3	0.94	1.87	99	0.99	2.73
4	0.99	2.64	167	1.42	3.92
5	1.13	3.85	241	0.96	2.66
6	1.07	3.71	247	0.96	2.66
7	1.05	3.35	219	0.87	2.42
8	1.12	3.57	219	0.83	2.23

Effects of Feeding Rate, Feeding Frequency, Stocking Density, Dietary Vitamin Level, and Kind of Binder on Growth

In Experiment II, the effects of several factors (Table 3) on the weight gain, FCE, and PER of *T. nilotica* were tested with 2 levels in a factorial design using an orthogonal L_8. The results of Experiment II are given in Tables 7, 8, and 9 and

Table 8. Analysis of variance for the weight gain, FCE, and PER data*1 from Experiment II

Data	Factor	df	V	F_0	F_0'	ρ (%)
Weight gain	A	1	19404	9.78	36.17**	81.84
	B	1	313	0.16	—	1.32
	A×B	1	313	0.16	—	1.32
	C	1	61	0.03	—	0.26
	D	1	13	<0.01	—	0.05
	E	1	1625	0.82	3.03	6.85
	e	1	1985			8.37
FCE	A	1	0.195	2.16	10.0*	52.9
	B	1	0.009	0.10	—	2.4
	A×B	1	0.004	0.04	—	1.0
	C	1	<0.001	<0.01	—	0.1
	D	1	0.001	0.01	—	0.3
	E	1	0.074	0.82	3.5	19.8
	e	1	0.090			24.2
PER	A	1	1.45	2.04	8.9*	51.9
	B	1	0.07	0.11	—	2.8
	A×B	1	0.03	0.04	—	1.2
	C	1	<0.01	<0.01	—	0.1
	D	1	<0.01	0.01	—	0.5
	E	1	0.56	0.79	3.1	18.1
	e	1	0.70			25.5

*1 Abbreviations used are as follows: V, variance; F_0, F-value; F_0', F-value when calculated with a pooled error; e, error; ρ, contribution ratio. Asterisks indicate a significant difference at $P<0.05$ (*) and $P<0.01$ (**).

Table 9. Effects of the factors on the weight gain, FCE, and PER of *T. nilotica* in Experiment II

Factor and level	Mean±confidence limit (P=0.95)		
	Weight gain (%)	FCE	PER
Feeding rate:			
A_1: 3.5%	133±42	1.22±0.21	3.36±0.60
A_2: 7.0%	232±42	0.91±0.21	2.51±0.60
Feeding frequency			
B_1: Twice/day	189±42	1.10±0.21	3.03±0.60
B_2: 3 times/day	176±42	1.03±0.21	2.84±0.60
Stocking density			
C_1: 30 fish/tank	180±42	1.06±0.21	2.93±0.60
C_2: 15 fish/tank	185±42	1.06±0.21	2.94±0.60
Vitamin level			
D_1: Halver's level	181±42	1.05±0.21	2.90±0.60
D_2: Halver's level×2	184±42	1.07±0.21	2.97±0.60
Kind of binder			
E_1: Agar	197±42	1.16±0.21	3.20±0.60
E_2: CMC	168±42	0.97±0.21	2.67±0.60

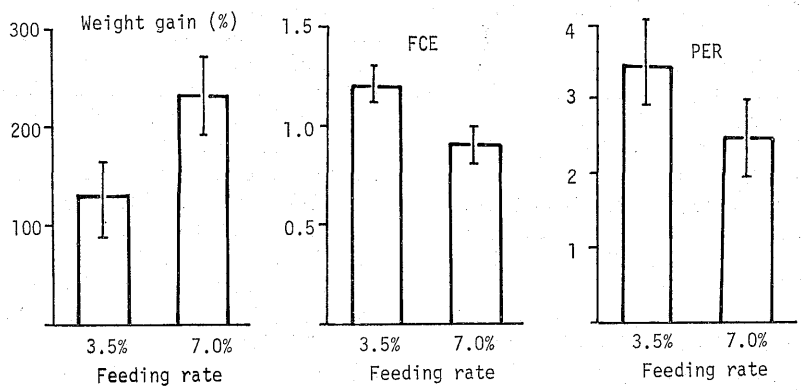

Fig. 2. Effects of feeding rate on the weight gain, FCE, and PER.
⊢—⊣; confidence limits (P=0.95)

Fig. 1. The growth of *Tilapia* was markedly different with the experimental conditions adopted (Table 7). The weight gain of *Tilapia* was highest in the group 6 reared under the following conditions; feeding rate (7% of total biomass/day), feeding frequency (twice/day), stocking density (15 fish/tank), dietary vitamin level (Halver's level[29]), and binder (CMC). The weight gain, FCE, and PER data were analysed by analysis of variance. As for the weight gain (Table 8), a significant difference ($P<0.05$) was observed in factor A but not in factors B, C, D, E, and A×B. Regarding the FCE and PER, a significant difference ($P<0.05$) was again detected in factor A but not in the other factors. Thus, the growth of *T. nilotica* was markedly affected by the feeding rate but not significantly by the feeding frequency, stocking density, dietary vitamin level, and kind of binder. Table 9 shows the effects of respective factors on the weight gain, FCE, and PER. The weight gain of *Tilapia* was higher when received the diet at the rate of 7% than at the rate of 3.5% (Table 9 and Fig. 2). As for the FCE and PER, however, the higher values were obtained by the rate of 3.5% than by the rate of 7%.

Viola and Arieli[30] have investigated the growth of *Tilapia* sp. in cages at 2 levels of protein (25% and 30%) and at 3 feeding rates (1.5%, 2.25%, and 3.0%) in a factorial design. They have shown that the higher protein diets improved the weight gain and feed conversion by 20% at each feeding level, and also that the increase in feeding rates brought forth in growth rates, but the response was diminished at the highest rate. Wang *et al.*[6] have demonstrated that daily feed consumption of *T. nilotica* decreased with an increase of protein levels

in diets, pointing out the necessity for maintaining a high digestible energy (DE) level even in a high protein diet not to lower a daily DE intake. Interestingly, they have further shown that daily feed consumption decreased with increasing cellulose levels in the case of a low (20%)-protein diet. As for the vitamin requirements, Kesamaru *et al.** have revealed that *T. nilotica* grew well on the diet supplemented the half amounts of vitamin mixture of Halver's recipe.[29] Although little studies have dealt with the effects of factors affecting the growth of *T. nilotica* in a well designed factorial experiment, we think that it may be desirable to assess the nutritional requirements of this fish in relation to the rearing conditions, especially feeding rates.

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