Effects of Dietary Protein, Lipid, and Digestible Carbohydrate Levels on the Weight Gain, Feed Conversion Efficiency, and Protein Efficiency Ratio of *Tilapia nilotica**

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Abstract

Feeding trials using purified diets, designed as 2-way layout experiments, were conducted to examine the effects of dietary protein (casein), lipid (a 1 to 1 mixture of soybean oil and pollack liver oil), and digestible carbohydrate (dextrin) on the growth of *Tilapia nilotica* in terms of weight gain, feed conversion efficiency, and protein efficiency ratios. *Tilapia* fingerlings grew best on diets containing 40% protein, 12% lipid and 30% digestible carbohydrate. When the dietary lipid level was fixed at 10%, good growth was obtained with 30% protein + 40% digestible carbohydrate, 40% protein + 30% digestible carbohydrate, and 30% protein + 30% digestible carbohydrate. The optimum digestible energy for *T. nilotica* was around 380-410 kcal/100 g diet. It also seems likely that *T. nilotica* utilizes lipids more effectively than digestible carbohydrates as energy sources when receiving the 35% protein-diets which meet the protein requirement. These results indicate that *T. nilotica* fingerlings grow optimally on deits containing 30-40% protein, 12-15% lipids, and 30-40% digestible carbohydrate.

Introduction

Tilapia has been intensively cultivated by fish-farmers in Japan since about 1970 using formula diets. However, most of the nutritional studies on any *Tilapia* species were concerned with supplemental feeding in extensive ponds (STICKNEY and HESBY, 1977; COLLINS and SMITHERMAN, 1978; MILLER, 1978). Several studies have investigated the protein requirements of *Tilapia* using test diets (DAVIS and STICKNEY, 1978; MAZID *et al.*, 1978, 1979; TESHIMA *et al.*, 1978; JAUNCEY, 1982; KESAMARU *et al.*,

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1982). In these studies, however, the protein requirements were determined using isocaloric diets with varying protein levels, whose energy levels were fixed arbitrarily with lipids and/or carbohydrates. The protein requirements of fish are likely to vary with the dietary levels of lipids and carbohydrates used as the major energy sources along with types of proteins used (ADRON *et al.*, 1976 ; GARLING and WILSON, 1976). In the present study, therefore, we intend to estimate the optimum dietary protein levels for *Tilapia nilotica* in relation to the lipid and carbohydrate levels in diets containing casein as a sole protein source. The feeding experiments were designed as two-way layout experiments (3 × 3 types) (ISHIKAWA *et al.*, 1967), and the results were evaluated statistically.

Materials and Methods

The fingerlings of *T. nilotica* were obtained from commercial *Tilapia* farms in Kagoshima (either Fuji-Enterprise or Shiroyama-gosan Co.) and maintained on a commercial carp ration for about 1 week prior to feeding trials using test diets. Three feeding experiments were conducted to clarify the effects of protein, lipid, and digestible carbohydrate levels in diets on growth of *T. nilotica*. In feeding trials, experimental groups were designed as two-way layout experiments, regarding dietary protein, lipid, and digestible carbohydrate levels as factors P, L, and C, respectively. The fingerlings were reared for 4 weeks in every feeding trial under the conditions given in Table 1. In Experiments I and II, feeding trials were replicated.

Table 1. Experimental conditions on the feeding trials of *T. nilotica*

Condition	Experiment-I		Experiment-II		Experiment-III
	Trial-1	Trial-2	Trial-1	Trial-2	Trial-1
Date of experiment	August	December	October	December	October
Feeding period (weeks)	4	4	4	4	4
Initial body weight (g)	1.29	1.60	0.88	1.58	0.88
Number of fish/tank (30 *l*)	15	12	15	12	15
Feeding level of diets*1	7%	7%	7%	7%	7%
Daily feeding frequency*2	Twice	Twice	Twice	Twice	Twice
Water temperature (°C)	27-29	25-27	27-29	25-27	26-28

*1 The basal ration of test diets was similar to that of the purified diet for *T. zillii* used in the previous study (TESHIMA *et al.*, 1978). Test diets contained the following ingredients (% of dry diets) : casein (20, 30 or 40), dextrin (10, 20, 30 or 40), pollack liver oil-soybean oil (1 : 1, w/w) (3, 4, 9, 11, 14 or 19), linoleic acid (1.0), L-tryptophan (0.5), L-methionine (0.5), minerals (4.0), vitamins (1.0), agar (3.0), and α-cellulose (equal to 100 %). The pH value of diets was adjusted to 7.0.

*2 The diets were given to the fingerlings at about 8 : 00 and 15 : 00 o'clocks every day.

The base ration of test diets was similar to that of the purified diets for *T. zillii* (TESHIMA *et al.*, 1978). The test diets contained varying levels of protein, lipid, and/or digestible carbohydrate using casein, pollack liver oil-soybean oil (1 : 1), and dextrin; respectively. The composition of the test diets appears in Tables 1, 2, 4, and 6. The methods for preparing the feed and of rearing the fingerlings were described previously (TESHIMA *et al.*, 1978).

The diets were evaluated in terms of body weight gain (%), feed conversion efficiency (FCE), and protein efficiency ratio (PER). FCE is the weight gain (g) of fish divided by the weight (g) of dry feed intaked. PER is the weight (g) of fish divided by the weight (g) of protein intaked. The feeding trial data were analyzed using analysis of variance. The effects of dietary protein, lipid, and carbohydrate levels, and their interactions on weight gain, FCE, and PER were evaluated statistically.

Digestible energy (DE) levels were provisionally calculated by using the following values; protein 4.5, lipids 9.0, and digestible carbohydrate 4.0 kcal/g.

Results

In Experiment I, the effects of dietary protein and lipid levels on the growth of *T. nilotica* were examined with 9 diets containing varying levels of protein (20%, 30%, and 40%) and lipid (4%, 12%, and 20%) and with a fixed digestible carbohydrate level of 30%. Tables 2 and 3 and Fig. 1 show the results of Experiment I. The analysis of variance (Table 3) showed that the weight gain of *T. nilotica* varied significantly ($P<0.01$) with both dietary protein and lipid levels examined. The FCE and PER of diets were also significantly ($P<0.01$) different with both dietary protein and lipid levels. A significant difference was not detected in the interaction between protein and lipid levels for weight gain, FCE and PER. Fig. 1 shows the effects of dietary protein and lipid levels on the weight gain, FCE, and PER in *T. nilotica*. The weight gain and FCE increased with increasing levels of protein from 20% to 40% and lipid from 4% to 12%. However, further increases in lipid levels from 12% to 20% did not show a significant improvement of weight gain and FCE. The PER decreased significantly as the dietary protein levels were increased, whereas it increased slightly with the increasing lipid levels in the diets. This indicates that dietary protein could be utilized more efficiently in a high-lipid diet than in a low-lipid diet, suggesting a protein-sparing effect of lipids. From the results of Experiment I, *T. nilotica* is likely to obtain optimum growth on the diets containing 40% protein and 12% lipid when the dietary digestible carbohydrate level is fixed at 30%.

In Experiment II, the dietary lipid level was fixed at 10%, and the effects of dietary protein and digestible carbohydrate levels on growth of *T. nilotica* were examined with 9 diets containing varying levels of protein (20%, 30%, and 40%). The results of Experiment II are given in Tables 4 and 5, and Fig. 2 and 3. The analysis of variance for the data from Experiment II (Table 5) showed that the weight gain of *Tilapia* varied significantly ($P<0.01$) with both dietary protein and digestible carbohydrate levels examined. The FCE varied with both dietary protein and digestible carbohydrate levels significantly at the 10% level but not significantly at the 5% level. The PER was significantly ($P<0.01$) different with dietary protein levels but not with the digestible carbohydrate levels examined. The interaction between protein and digestible carbohydrate levels was not significant with the FCE and PER data but significant ($P<0.01$) with the weight gain data. Fig. 2 show the effects of dietary protein and digestible carbohydrate levels on weight gain, FCE, and PER which were estimated without regard to the interaction between protein and digestible carbohydrate levels. The FCE was improved with increasing protein levels from 20% to 40% and also with the increasing digestible carbohydrate levels from 20% to 40%, but it did not vary significantly with the increase in digestible carbohydrate levels from 30% to 40%.

Table 2. Results of Experiment-I. The fish were fed the diets containing different levels of protein (P) and lipid (L) at the fixed digestible carbohydrate (C) level of 30 % for 4 weeks.

Exptl. group	Level (%)*1			DE (kcal/ 100 g)*2	Weight gain (%)	Feed efficiency*3	PER*4
	P	L	C				
1	20	4	30	246	132*5	0.69	3.43
					142*5	0.64	3.26
2	20	12	30	318	165	0.83	4.13
					168	0.72	3.52
3	20	20	30	390	166	0.71	3.56
					187	0.77	3.85
4	30	4	30	291	178	0.89	2.90
					224	0.86	2.90
5	30	12	30	363	223	0.99	3.34
					256	0.99	3.33
6	30	20	30	435	267	0.99	3.30
					278	1.02	3.43
7	40	4	30	336	190	0.87	2.16
					240	0.89	2.21
8	40	12	30	408	309	1.27	3.19
					272	1.02	2.53
9	40	20	30	480	256	1.12	2.77
					275	1.06	2.64

*1 The dietary protein and lipid levels were adjusted with casein and pollack liver oil-soybean oil (1 : 1), respectively.

*2 Provisional digestive energy (DE) : protein, 4.5 kcal/g ; lipid, 9.0 kcal/g ; digestible carbohydrate, 4.0 kcal/g.

*3 Feed conversion efficiency = g gain/g feed.

*4 Protein efficiency ratio = g gain/g protein intaked.

*5 The data were obtained by trials 1 (upper) and 2 (lower).

Table 3. Analysis of variance*1 with the data from Experiment-I

Data	Factor	SS	df	V	Fo
Weight gain	P	65001.4	2	32500.7	48.50**
	L	21525.6	2	10762.9	16.06**
	PxL	4581.1	4	1145.3	1.71
	R	2678.9	1	2679.0	4.00
	e	5361.0	8	670.1	
Feed efficiency	P	3103.9	2	1552.1	39.43**
	L	991.0	2	495.5	12.59**
	PxL	173.4	4	43.5	1.10
	R	75.9	1	75.9	1.93
	e	314.7	8	39.4	
PER	P	3.635	2	1.818	42.20**
	L	1.031	2	0.517	12.00**
	PxL	0.070·	4	0.018	0.43
	R	0.055	1	0.055	1.29
	e	0.344	8	0.043	

*1 Abbreviations used: P, protein levels; L, lipid levels; PxL, interaction between factors P and L; R, variability of data between the feeding trials; e, error; SS, sum of squares; df, degree of freedom; V, variance; Fo, variance ratio. Two asterisks (**) indicate a statistically significant difference ($P<0.01$).

As for the weight gain data, a significant interaction was detected between protein and digestible carbohydrate levels (Table 5). This implies that the effects of dietary protein and digestible carbohydrate levels on the weight gain of *T. nilotica* were variable with the dietary level of a counterpart of each other. Fig. 3 shows the effects of dietary protein and digestible carbohydrate levels on weight gains which were estimated in consideration of the interaction. The weight gain of *Tilapia* increased as dietary proein levels were increased from 20% to 40% in the case of the diets containing 20% and 30% levels of digestible carbohydrate. As for the 40% carbohydrate-diets, the weight gain of *Tilapia* increased with increasing protein levels from 20% to 30%, but decreased at the 40% protein level. The effect of digestible carbohydrate levels in diets resembled that of dietary protein levels. The increases in digestible carbohydrate levels from 20% to 40% resulted in improvement of weight gain, except for the case of the diet containing 40% protein and 40% carbohydrate. In Experiments II, the highest weight gain was apparently obtained on the diet containing 30% protein and 40% digestible

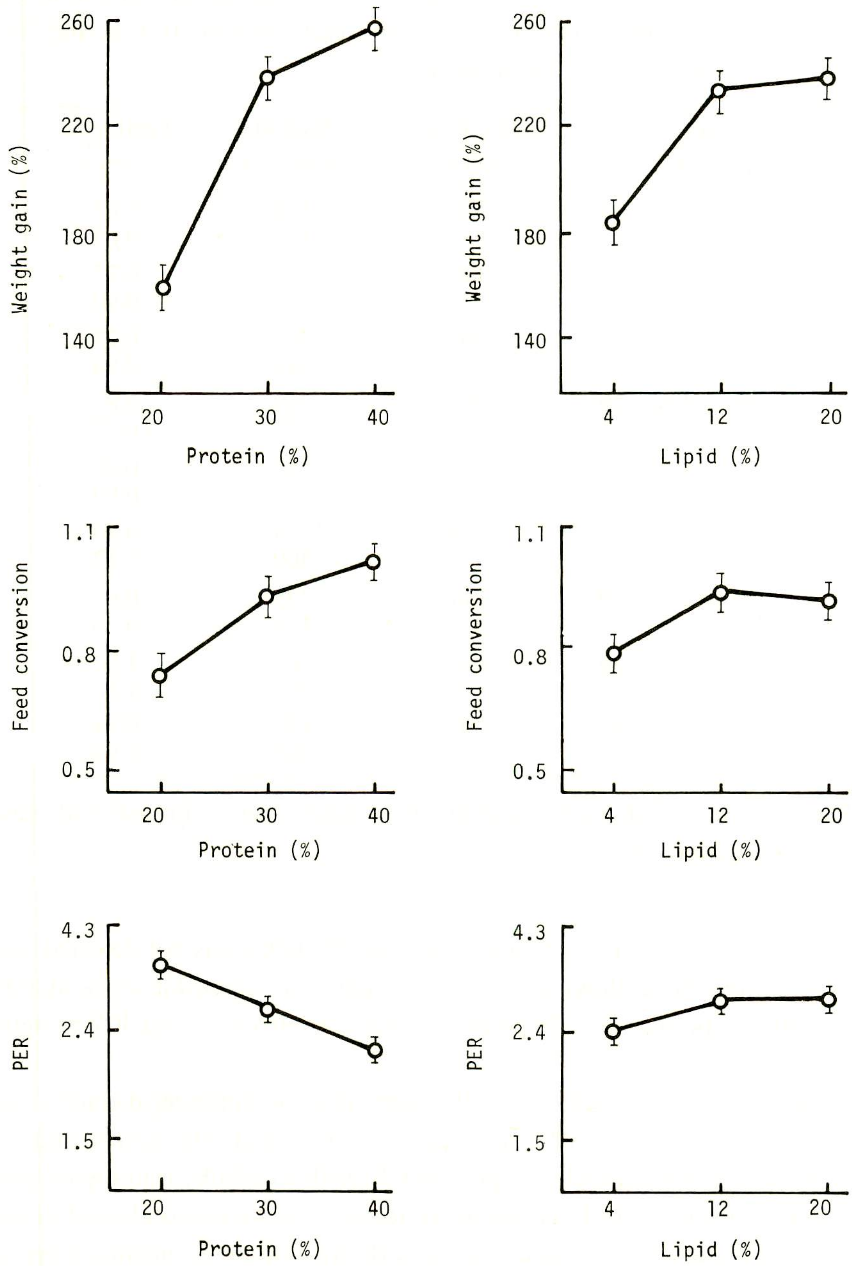

Fig. 1. The effects of dietary protein and lipid levels on growth of *T. nilotica*. The means and confidence limits (P=0.95) for weight gain, feed conversion efficiency, and protein efficiency ratio were estimated from the data of Experiment-I.

Table 4. Results of Experiment-II. The fish were fed the diet containing different levels of protein (P) and digestible carbohydrate (C) at the fixed lipid (L) level of 10.0% for 4 weeks.

Exptl. group	Level (%)*1 P	L	C	DE (kcal/100 g)	Weight gain (%)	Feed efficiency	PER
21	20	10	20	260	129 162	0.61 0.69	3.07 4.72
22	20	10	30	300	167 235	0.73 0.90	3.66 4.55
23	20	10	40	340	205 242	0.77 0.94	4.35 3.59
24	30	10	20	305	210 173	0.87 0.72	2.90 2.30
25	30	10	30	345	243 258	0.96 0.99	3.20 3.26
26	30	10	40	385	216 300	0.89 1.12	2.93 3.73
27	40	10	20	350	235 178	0.97 0.76	2.43 1.87
28	40	10	30	390	262 296	1.02 1.36	2.52 3.00
29	40	10	40	430	197 207	0.84 0.84	2.10 2.13

*1 The protein and digestible carbohydrate levels were adjusted with casein and dextrin, respectively.

carbohydrate. However, a significant difference ($P<0.05$) was not detected among the 3 diets containing the following levels of protein and digestible carbohydrate; 30% protein + 40% carbohydrate, 40% protein + 30% carbohydrate, and 30% protein + 30% carbohydrate.

The results of Experiments I and II suggest that the optimum dietary protein level for *T. nilotica* is about 30-40%, although it varies with the levels and kinds of non-protein energy souces such as lipids and digestible carbohydrates when casein was used as a protein source. In Experiment III, feeding trials were conducted to clarify the availability of dietary lipids and digestible carbohydrate by *T. nilotica* when the diets contained sufficient protein for meeting the protein requirement. *T. nilotica* fingerlings were fed 9 diets containing varying levels of lipids (5%, 10%, and 15%) and digestible carbohydrate (20%, 30%, and 40%) at a fixed protein levels of 35%. The results of Experiment III are shown in Tables 6 and 7, and Fig. 4. The analysis of variance (Table 7) showed that the weight gain of *T. nilotica* was significantly different with dietary lipids ($P<0.01$) and digestible carbohydrate ($P<0.05$) levels. The FCE and PER were also significantly different with the lipid levels examined ($P<0.01$) but not with the digestible carbohydrate levels. Fig. 4. shows the effects of dietary lipid and

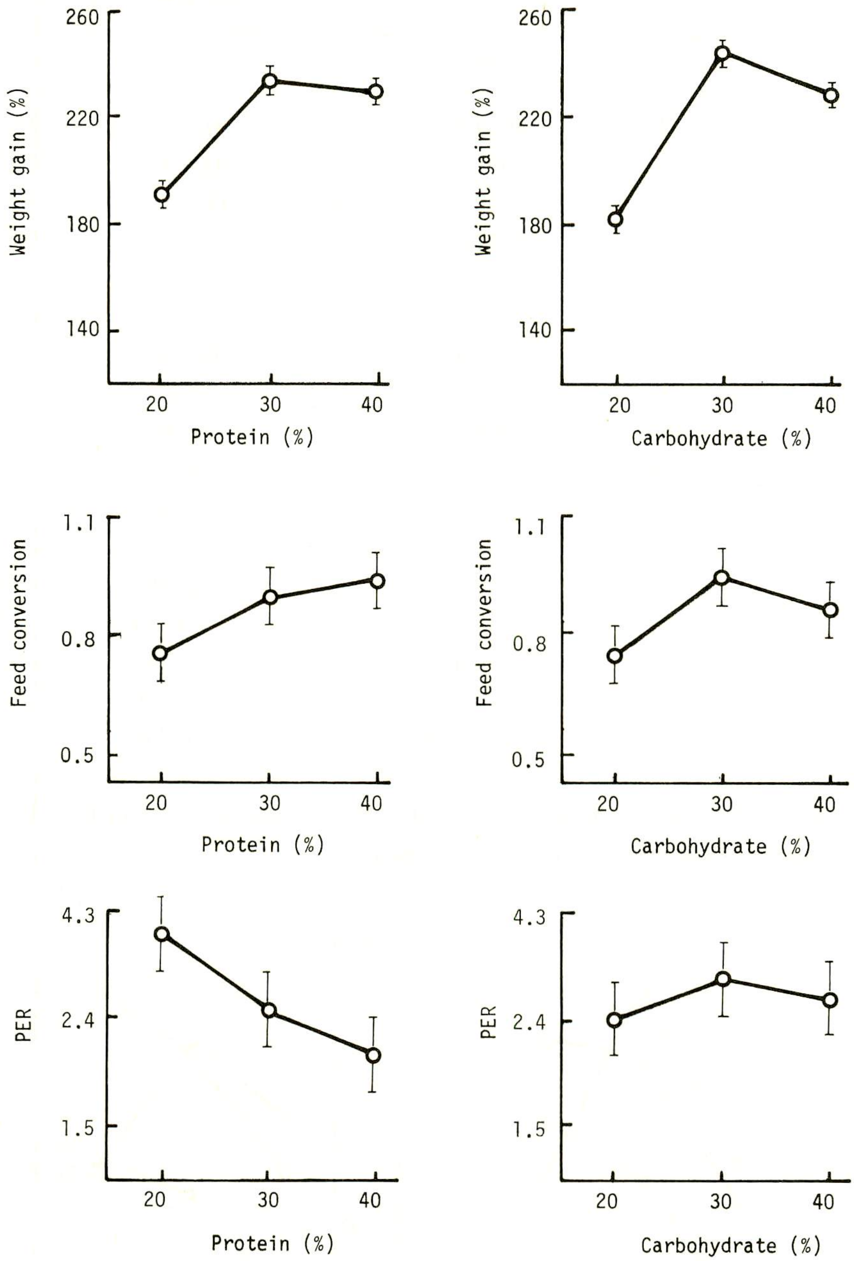

Fig. 2. The effects of dietary protein and digestible carbohydrate levels on growth of *T. nilotica.* The means and confidence limits (P=0.95) for weight gain, feed conversion efficiency, and protein efficiency ratio were estimated from the data of Experiment-II.

Table 5. Analysis of variance with the data from Experiment-II

Data	Factor*1	SS	df	V	Fo
Weight gain	P	14059.5	2	7030.0	115.5**
	C	34001.9	2	17001.1	279.2**
	PxC	13538.8	4	3384.8	55.6**
	R	12119.0	1	12119.0	199.0**
	e	487.5	8	60.9	
Feed efficiency	P	1203.5	2	601.9	3.21
	C	1493.8	2	747.0	3.99
	PxC	1000.0	4	250.9	1.34
	R	230.6	1	230.6	1.23
	e	1498.6	8	187.4	
PER	P	7.981	2	3.991	12.09**
	C	0.826	2	0.414	1.37
	PxC	0.439	4	0.111	0.34
	R	0.256	1	0.256	0.78
	e	2.645	8	0.330	

*1 C, digestible carbohydrate levels ; PxC, interaction between factors P and C. See Table 3 for other abbreviations used.

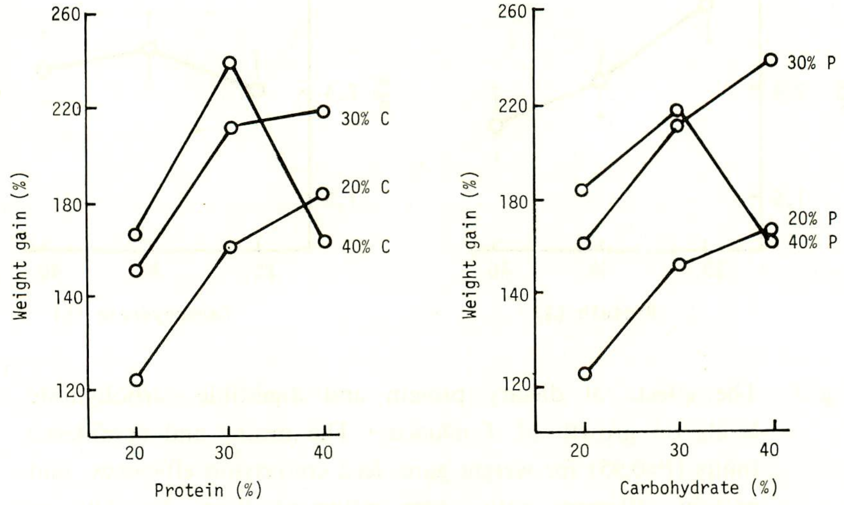

Fig. 3. The effects of dietary protein and digestible carbohydrate levels on weight gain of *T. nilotica*, which were evaluated from the data of Experiment-II in consideration of the interaction PxC.

Table 6. Results of Experiment-III. The fish were fed the diets containing different levels of lipid (L) and digestible carbohydrate (C) at the fixed protein (P) level of 35 % for 4 weeks.

Exptl. group	Level (%)*1			DE (kcal/ 100 g	Weight gain (%)	Feed efficiency	PER
	P	L	C				
31	35	5	20	283	84	0.44	1.27
32	35	10	20	328	163	0.74	2.13
33	35	15	20	373	215	0.93	2.64
34	35	5	30	323	108	0.47	1.36
35	35	10	30	368	193	0.86	2.47
36	35	15	30	413	250	0.99	2.82
37	35	5	40	363	126	0.61	1.76
38	35	10	40	408	199	0.87	2.49
39	35	15	40	453	228	0.96	2.75

*1 The lipid and digestible carbohydrate levels were adjusted with pollack liver oil-soybean oil (1 : 1) and dextrin, respectively.

Table 7. Analysis of variance with the data from Experiment-III

Data	Factor	SS	df	V	Fo*1
Weight gain	C	3691.4	2	1845.8	7.88*
	L	49163.1	2	24581.6	104.91**
	e	937.5	4	234.3	
Feed efficiency	C	183.7	2	91.8	3.61
	L	3183.4	2	1591.8	62.62**
	e	101.9	4	25.4	
PER	C	0.096	2	0.049	1.04
	L	2.582	2	1.290	27.39**
	e	0.191	4	0.047	

1 Asterisks indicate significant differences (, $P<0.05$; **, $P<0.01$)

digestible carbohydrate levels on the weight gain, FCE, and PER. The weight gain improved with the increase in lipid levels from 5% to 15% and also with the increase in digestible carbohydrate levels from 20% to 30%. But the increase in digestible carbohydrate levels from 30% to 40% did not result in a significant increase in weight gains. The increase in lipid levels from 5% to 10% or 15% was effective in improving

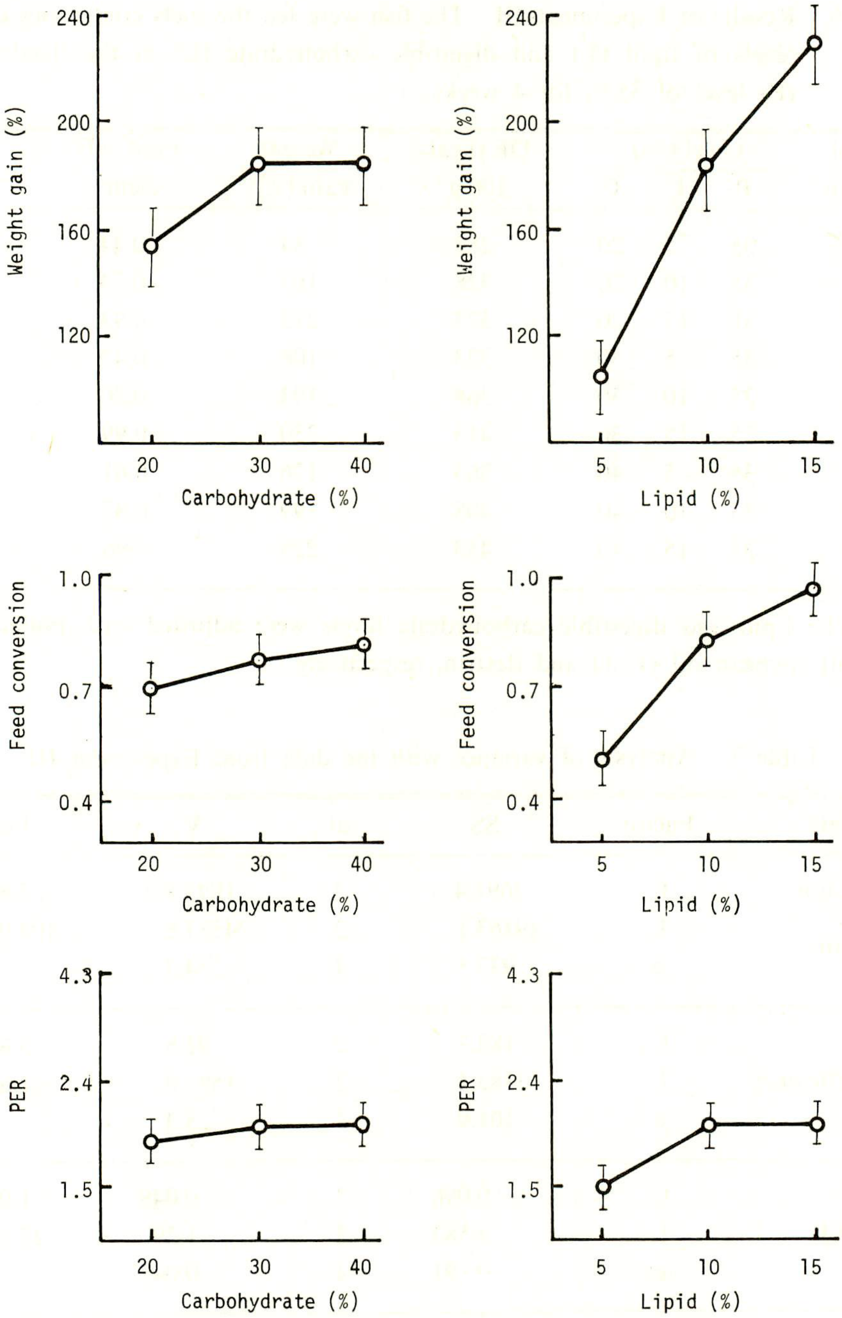

Fig. 4. The effects of dietary lipid and digestible carbohydrate levels on growth of *T. nilotica*. The means and confidence limits (P=0.95) for weight gain, feed conversion efficiency, and protein efficiency ratio were estimated from the data of Experiment-III.

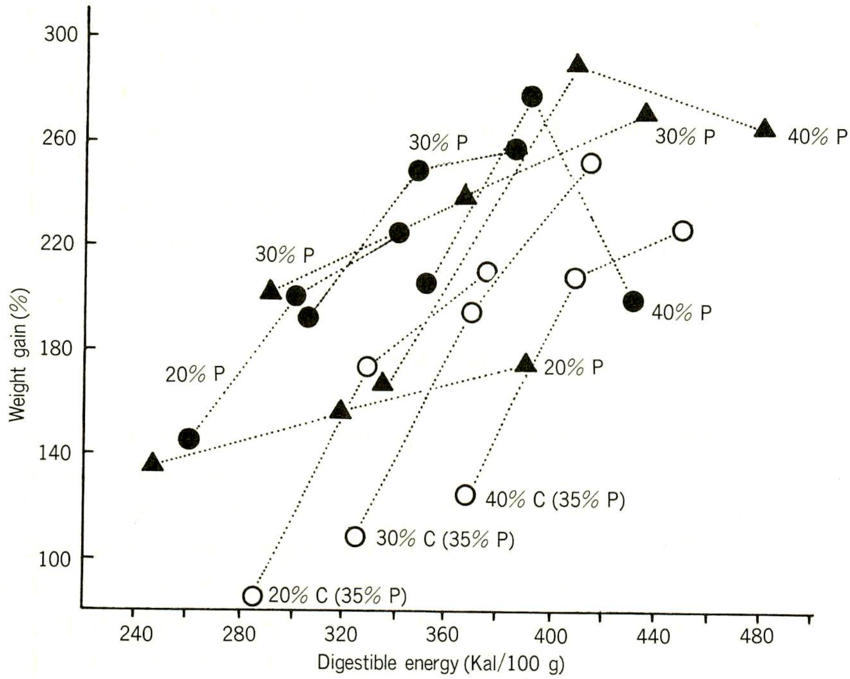

Fig. 5. The effect of digestible energy levels in diets on weight gain of *T. nilotica*. The marks ▲, ●, and ○ in the figure shows the data obtained from Experiments I, II, and III, respectively. P and C indicate dietary protein and digestible carbohydrate levels, respectively.

the FCE and PER, whereas that in digestible carbohydrate levels from 20% to 40% had no significant effect on these parameters. This suggests that *T. nilotica* possibly utilizes lipids more effectively than digestible carbohydrates as energy sources in the 35 % protein-diet which probably meets the protein requirements of this fish. The results of Experiment III show that *T. nilotica* probably obtains the optimum growth on diets containing 15 % lipid and 30 % digestible carbohydrate when the dietary protein level is fixed at 35 %.

Fig. 5. shows the relationship between DE (kcal/100 g diet) and weight gain (%) of *T. nilotica*. The effects of DE and DE/P ratio [DE (kcal/kg)/ protein (%)] on the weight gain varied with dietary protein levels. In the 20% protein-diets, the weight gain improved with increasing DE levels, but the growth of *Tilapia* was very poor as compared with the diets containing higher protein levels. In the 30% protein-diets, the weight gain also improved with the increasing DE levels and showed the highest value at a DE level of 385 (diet 26, DE/P = 128) and 435 (diet 6, DE/P = 145). In the 35% protein-diets, the highest weight gain was attained at a DE level of 413 (diet 36, DE/P = 118). In the 40% protein diets, the highest weight gain was obtained at a DE level of 390 (diet 28, DE/P = 97.5) and 408 (diet 8, DE/P = 102), however it decreased slightly (diet 9) or markedly (diet 29) when the DE levels exceed more than 430. These

results show that the optimum DE levels are about 380–410 kcal/100 g when diets contain the minimum amount of protein to satisfy the protein requirement of *T. nilotica*. Also, the present study suggests that the optimum DE/P ratio for *T. nilotica* is likely to decrease with the increasing protein levels in diets from 30% to 40%.

Considering the results of Experiments I, II and III together, it is concluded that *T. nilotica* grows best when the diets contain 30-40% protein, 12-15% lipid, and 30-40% digestible carbohydrate under the conditions adopted in the present study.

Discussion

Tilapia are generally regarded as herbivorous and omnivorous fish. In fact, HOUSER (1975) and BAYNE *et al*. (1976) have shown that vegetable proteins could be used in large quantities successfully in *Tilapia* diets. DAVIS and STICKNEY (1978) have demonstrated that *T. aurea* grew well on diets with soybean meal as the protein source. As for *Sarotherodon mossambicus,* some plant proteins have been revealed to be usable in diets as partial substitutes for fish meal (JACKSON *et al*., 1982). In the case of *T. nilotica,* however, KESAMARU and MIYAZONO (1978a) have shown that the dietary value of soybean meal was inferior to that of animal proteins such as a white fish meal, brown fish meal, and silkworm pupa meal. Interestingly, they have demonstrated that a wheat germ protein had a high dietary value comparable to white fish meal for *T. nilotica,* suggesting that the optimum dietary protein levels for this fish is about 40% when a white fish meal was used as a sole protein source (KESAMARU and MIYAZONO, 1978a, 1978b).

Apart from the above studies using formula diets composed of natural ingredients, the protein requirements of *Tilapia* species have been investigated by several groups of workers using the purified diets containing casein as a sole protein source. Previously we have shown that the optimum dietary protein level for *T. zillii* was 35-40% (TESHIMA *et al*., 1978). MAZID *et al*. (1979) have also revealed that the same species of *Tilapia* required about 35% protein for optimum growth while 30% protein for maximum body protein deposition. KESAMARU *et al*. (1982) have demonstrated that *T. nilotica* showed the best growth on a diet with 40% protein. In these studies using purified diets, however, the optimum dietary protein levels were determined by one-way layout experiments without regard to the variabilities of experimental data. Also, only a little information on the relationship between the protein requirements and dietary energy levels in *T. nilotica* is available* .

In the present study, the optimum dietary protein level for growth of *T. nilotica* was examined using purified diets containing casein as a sole protein source in relation to the dietary energy levels which were altered with either lipids or digestible carbo-

* While this work was in press, WANG *et al*. (1985a, 1985b) published a paper on the optimum dietary protein and digestible energy levels for *T. nilotica*.

hydrate, and the data obtained were statistically evaluated. Growth of *T. nilotica* improved with increasing protein and lipid levels irrespective of one another's level when the diets contained 30% digestible carbohydrate. Whereas, the effects of dietary protein and digestible carbohydrate levels on growth were affected with the levels of a counterpart of each other when dietary lipid levels were fixed at 10%. The increase in protein levels from 20% to 30% and digestible carbohydrate levels from 20% to 30% resulted in a marked improvement of growth. However, further increases in protein levels from 30% to 40% and digestible carbohydrate levels from 30% to 40% did not show a marked improvement of growth. Particularly, growth of *T. nilotica* was suppressed when fed the 40% protein-diet containing higher levels of non-protein energy sources such as 40% digestible carbohydrate and 10% lipids. The optimum dietary protein levels for *T. nilotica* thus varied with the kind and dietary level of non-protein energy sources, lipids and carbohydrate. In the present study, the optimum dietary protein level for *T. nilotica* fingerlings was estimated to be 30-40% in diets when casein was used as a sole protein source and sufficient energy was provided with soybean oil-pollack liver oil (1 : 1) and dextrin. The optimum dietary protein levels for *T. nilotica* obtained in the present study are apparently similar to those reported for *Tilapia* species which had been assessed using casein (TESHIMA *et al.*, 1978 ; MAZID *et al.*, 1979 ; KESAMARU *et al.*, 1982) and other protein sources (DAVIS and STICKNEY, 1978 : WINFREE and STICKNEY, 1981 ; VIOLA and ARIELI, 1982 ; JAUNCEY, 1982).

The present study also showed that the weight gain of *T. nilotica* improved with increasing DE levels, indicating that the optimum level was around 380-410 kcal/100 g. This value was slightly lower than the optimum DE level for the rainbow trout *Salmo gairdneri* (TAKEUCHI *et al.*, 1978) and was higher than that for the carp *Cyprinus carpio* (TAKEUCHI *et al.*, 1979).

MAZAID *et al.* (1978) have shown that *T. zillii* required the same 10 amino acids reported to be essential for other fish. JACKSON *et al.* (1982) have also pointed out that the nutritive value of dietary protein for *S. mossambicus* was related to the content of essential amino acids. Moreover, BOWEN (1980) has repoted that *S. mossambicus* in Lake Valencia, Venezuela, grew rapidly on a low-protein diet by assimilating detrital non-protein amino amino acids. The above information supposes that the protein requirements of *Tilapia* are variable with the quality of protein sources used. Accordingly, we think that the protein requirements of *Tilapia* should be examined in further detail in the future in relation to the amino acid pattern of dietary proteins along with the kind and level of non-protein energy sources. To obtain a reliable answer to these question, it may desirable to replicate the feeding experiments in a factorial design.

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