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# Optimum Protein Levels in Casein-Gelatin Diets for *Tilapia nilotica* Fingerlings*1

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## Abstract

Feeding trials using purified diets, designed as two-way layout experiments, were performed to clarify the optimum dietary protein level for *Tilapia nilotica* fingerings when they were reared with casein-gelatin (3 : 1) diets with two digestible carbohydrate levels (30% and 40%). Regardless the digestible carbohydrate levels examined, the weight gain and feed conversion efficiency (FCE) of the fingerlings improved with increasing protein levels from 25% to 35%, whereas the protein efficiency ratio (PER) leveled off within the range of 25% to 35% protein levels and decreased with increase of protein levels from 35% to 40%. The digestible carbohydrate levels had no significant effects on the weight gain, FCE, and PER of *T. nilotica.* These results indicate that *T. nilotica* fingerlings grow optimally on diets containing about 35% protein when diets contain 12% lipids and 30—40% digestible carbohydrate.

Several workers have shown the optimum dietary protein level for *Tilapia nilotica* fingerlings using a casein as a protein source.[1-4] Previously, we have demonstrated that the fingerling grew optimally on diets containing 30—40% protein, 12—15% lipids, and 30—40% digestible carbohydrate when casein was used as a protein source.[5] Later, we have also revealed that the fingerlings probably grew better on diets containing casein-gelatin (3 : 1) and casein with essential amino acid supplements than on a casein diet.[6] Although it was unsuccessful to detect a significant difference ($P<0.05$) among these diets possibly due to a few trials, casein thus seemed unlikely to be the best protein source for *T. nilotica.* In the present study, therefore, the feeding trials were conducted to get more information on the nutritive value of casein-gelatin diets. The object of this work is also to estimate the optimum protein levels in casein-gelatin diets with two levels of digestible carbohydrate (30% and 40% dextrin).

## Materials and Methods

The fingerlings of *T. nilotica* were obtained from Fuji-Enterprise in Kagoshima and

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maintained on a commercial carp ration for 1 week until used. In feeding trials, experimental groups were designed as two-way layout experiments, regarding dietary protein and digestible carbohydrate levels as factors P and C, respectively. Protein and digestible carbohydrate sources were casein-gelatin (3 : 1) and dextrin, respectively. The fingerlings were reared in duplicate 30-$l$ tanks for each test diet under the conditions given in Table 1 using eight test diets (Table 2) containing varying levels of proteins (25%, 30%, 35%, and 40%) and digestible carbohydrate (30% and 40%) and with a fixed lipid level of 12%. Lipid sources were 11% pollack liver oil-soybean oil (1 : 1) and 1% linoleic acid. The base ration of test diets was the same as reported previously.[5,6] The methods for preparing the feed and of rearing the fingerlings were similar to those described previously.[1] Weight gain, feed conversion efficiency (FCE), and protein efficiency ratio (PER) data were statistically evaluated by analysis of variance.

Table 1. Rearing conditions of *Tilapia*

| Condition | Remark |
|---|---|
| Feeding period | 4 weeks (Nov. 1 to 28) |
| Average initial body wt. | 0.56 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 | 29°C |

Table 2. Experimental groups and the composition of test diets

| Exptl. group*1 | Test diet | Composition (%)*2 | | | DE (kcal/100g)*4 | DE/P*5 |
|---|---|---|---|---|---|---|
| | | Protein | Carbohydrate*3 | Lipid | | |
| 1 | 1 | 25 | 30 | 12 | 340.5 | 136 |
| 2 | 2 | 30 | 30 | 12 | 363.0 | 121 |
| 3 | 3 | 35 | 30 | 12 | 385.5 | 110 |
| 4 | 4 | 40 | 30 | 12 | 408.0 | 102 |
| 5 | 5 | 25 | 40 | 12 | 380.5 | 152 |
| 6 | 6 | 30 | 40 | 12 | 403.0 | 134 |
| 7 | 7 | 35 | 40 | 12 | 425.5 | 121 |
| 8 | 8 | 40 | 40 | 12 | 448.0 | 112 |

*1 The feeding trial was conducted in the duplicate tanks (30 $l$) with each test diet.
*2 Casein-gelatin (3 : 1) and dextrin were used as proteins and digestible carbohydrate sources, respectively. The base ration of test diets was as follows: Casein-gelatin (3 : 1), 25%, 30%, 35%, or 40%; dextrin, 30% or 40%; pollack liver oil-soybean oil (1 : 1), 11%; linoleic acid, 1%; minerals, 4%; vitamins, 1%; agar, 3%; $\alpha$-cellulose (equal to 100%).
*3 Digestible carbohydrate.
*4 Digestible energy (DE) levels were provisionally calculated using the following values; protein 4.5, lipid 9.0, and digestible carbohydrate 4.0 kcal/g.
*5 DE/P: Digestible energy (kcal/kg)/protein (%).

## Results

Table 3 shows the results of feeding experiment. The analysis of variance (Table 4)

Table 3. Results of feeding experiment

| Exptl. group | Weight gain (%) | | FCE*[1] | | PER*[2] | |
|---|---|---|---|---|---|---|
| | Tank-1 | Tank-2 | Tank-1 | Tank-2 | Tank-1 | Tank-2 |
| 1 | 176 | 146 | 0.72 | 0.64 | 2.87 | 2.72 |
| 2 | 195 | 239 | 0.79 | 0.90 | 2.62 | 3.02 |
| 3 | 283 | 283 | 0.97 | 1.00 | 2.77 | 2.85 |
| 4 | 290 | 258 | 0.96 | 0.92 | 2.40 | 2.30 |
| 5 | 177 | 136 | 0.72 | 0.61 | 2.86 | 2.59 |
| 6 | 206 | 205 | 0.82 | 0.82 | 2.72 | 2.72 |
| 7 | 253 | 246 | 0.93 | 0.93 | 2.65 | 2.66 |
| 8 | 304 | 248 | 1.03 | 0.93 | 2.45 | 2.33 |

*[1] Feed conversion efficiency : g gain/g feed
*[2] Protein efficiency ratio : g gain/g protein intake

Table 4. Analysis of variance for weight gain, FCE, and PER data *[1]

| Data | Factor*[2] | df | V | $F_0$ | $F_0'$ |
|---|---|---|---|---|---|
| Weight gain | P | 3 | 11664.2 | 23.80** | 24.81** |
| | C | 1 | 564.1 | 1.16 | |
| | PxC | 3 | 328.2 | 0.49 | |
| | R | 1 | 945.6 | 1.94 | |
| | e | 7 | 488.3 | | |
| FCE | P | 3 | 0.0739 | 26.77** | 32.77** |
| | C | 1 | 0.00076 | 0.27 | |
| | PxC | 3 | 0.00157 | 0.57 | |
| | R | 1 | 0.00226 | 0.82 | |
| | e | 7 | 0.00276 | | |
| PER | P | 3 | 0.1485 | 7.34* | 9.71** |
| | C | 1 | 0.0203 | 1.00 | |
| | PxC | 3 | 0.0067 | 0.33 | |
| | R | 1 | 0.0014 | 0.07 | |
| | e | 7 | 0.0202 | | |

*[1] Abbreviations used are as follows : df, degree of freedom ; V, variance ; $F_0$, F-value ; $F_0'$, F-value when calculated with a pooled error ($V_{E'}$). The $V_{E'}$ values of weight gain, FCE, and PER data were 470.2, 0.00225, and 0.0153, respectively. Asterisks indicate a significant difference at $P<0.05$ (*) and $P<0.01$ (**).
*[2] P, protein level ; C, digestible carbohydrate level ; PxC, interaction between factors P and C ; R, variability of data between the data of duplicate tanks ; e, error.

showed that the weight gain, FCE, and PER of *T. nilotica* varied significantly ($P<0.01$) with dietary protein levels but not with dietary digestible carbohydrate levels. No significant difference ($P>0.05$) was detected with an interaction between protein and digestible carbohydrate levels. Figs. 1, 2 and 3 show the effects of dietary protein levels on the weight gain, FCE, and PER of *T. nilotica*, respectively. The weight gain improved with increasing protein levels from 25% to 35%, but not significantly from 35% to 40%. The FCE also improved with increasing protein levels from 25% to 35%, but not

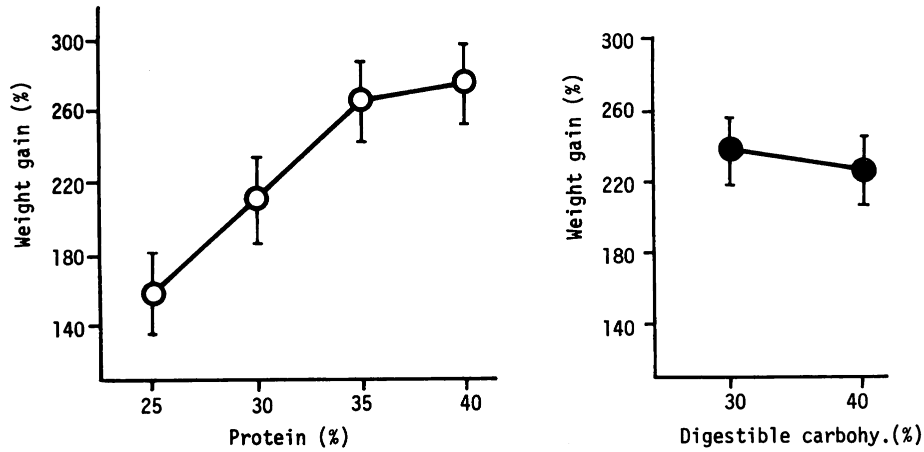


Fig. 1. Effects of dietary protein and digestible carbohydrate levels on weight gain of *Tilapia*. ⊢⊣, confidence limits (P =0.95)

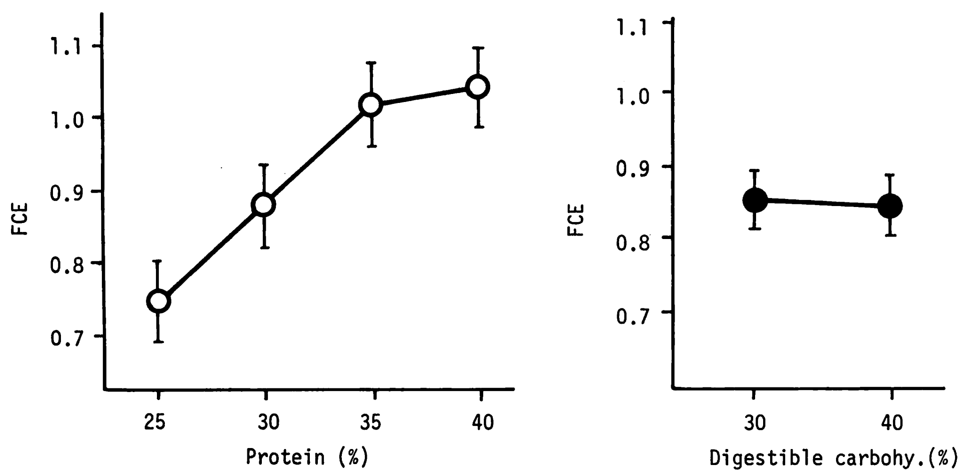


Fig. 2. Effects of dietary protein and digestible carbohydrate levels on feed conversion efficiency (FCE) of *Tilapia*. ⊢⊣, confidence limits (P =0.95)

significantly from 35% to 40%. The PER leveled off from 25% to 35% protein levels and decreased significantly with increasing protein levels from 35% to 40%. Whereas, the weight gain, FCE, and PER of *T. nilotica* did not improve significantly by the elevation of dietary digestible carbohydrate levels from 35% to 40%. Thus, *T. nilotica* fingerlings are likley to obtain the optimum growth on the diets containing less than 35 % protein regardless the carbohydrate levels (30% or 40%) when the diets contained 12% lipids.

Fig. 4 shows the relationship between the weight gain of *T. nilotica* and ratio of digestible energy (DE) to protein (P). The results indicate that the optimum ratio of DE/P was 100–110. Table 5 shows the data of the growth of *T. nilotica* in the present study and previous studies. The analysis of variance with the data revealed that a significant difference between

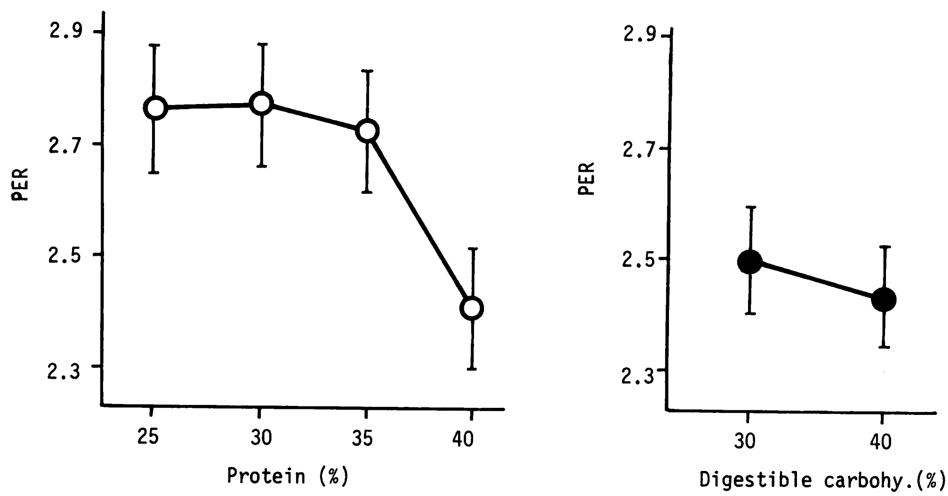


Fig. 3. Effects of dietary protein and digestible carbohydrate levels on protein efficiency ratio ( PER ) of *Tilapia*. ├─┤, confidence limits ( P =0.95)

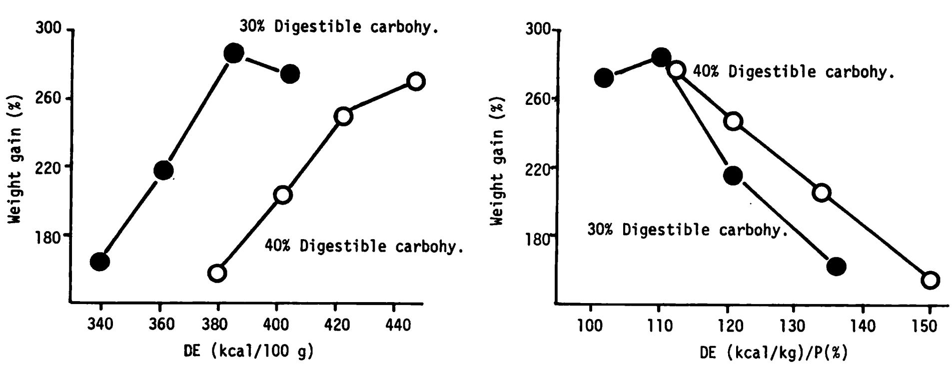


Fig. 4. Relationships between the weight gain of *T. nilotica* and either DE levels or DE/P ratios.

Table 5. Data of the growth of *T. nilotica* fingerlings reared with the casein-gelatin (3 : 1) diet and casein diet.

| Protein source | Weight gain (%) | FCE | PER | Dietary level (%)*1 | | | Data*2 |
|---|---|---|---|---|---|---|---|
| | | | | P | L | C | |
| Casein-gelatin (3:1) | 283 | 0.97 | 2.77 | 35 | 12 | 30 | Present study |
| | 283 | 1.00 | 2.85 | 35 | 12 | 30 | Present study |
| | 253 | 0.93 | 2.65 | 35 | 12 | 40 | Present study |
| | 246 | 0.93 | 2.66 | 35 | 12 | 40 | Present study |
| | 238 | 0.84 | 2.40 | 35 | 15 | 30 | Previous study[6] |
| | 243 | 0.94 | 2.70 | 35 | 15 | 30 | Previous study[6] |
| Mean*3 | 258±24 | 0.94±0.10 | 2.67±0.27 | | | | |
| Casein | 215 | 0.83 | 2.36 | 35 | 15 | 30 | Previous study[6] |
| | 174 | 0.66 | 1.90 | 35 | 15 | 30 | Previous study[6] |
| | 250 | 0.99 | 2.82 | 35 | 15 | 30 | Previous study[5] |
| | 228 | 0.96 | 2.75 | 35 | 15 | 40 | Previous study[5] |
| Mean*3 | 216±29 | 0.86±0.12 | 2.46±0.33 | | | | |

*1 P, protein; L, lipid (6.5% pollack liver oil, 6.5% soybean oil, and 1.0% linoleic acid); C, digestible carbohydrate (dextrin).

*2 Date were obtained in the present and previous studies. Except for the lipid and digestible carbhydrate levels, the compositions of other ingredients were similar each other in these feeding trials.

*3 Population means ± confidence limits (P=0.95). The analysis of variance showed that a significant difference ($P<0.05$) was detected only with the weight gain data.

the casein diet and casein-gelatin (3 : 1) was detected with the weight gain ($P<0.05$), but neither with the FCE nor the PER ($P>0.05$) (Table 5). *T. nilotica* fingerlings were thus found to grow better on the casein-gelatin (3 : 1) diet than the casein diet.

## Discussion

There have been several reports on the optimum dietary protein levels for *T. nilotica* when casein was used as a sole protein source.[1-3] In these studies, however, the optimum dietary protein levels have been estimated using diets, whose energy levels were arbitrarily fixed with lipids and/or carbohydrates, by one-way layout experiments without replications. Accordingly, one can not get the information on the certainty and limits of the conclusion obtained due to no data of variabilities of observations in feeding trials. In addition, some diets used in these studies were not well nutritionally formulated, especially on lipid sources. Recently, Wang *et al.*[4] have shown by casein diets containing corn oil, a lipid with the high nutritive value for *T. nilotica*,[7,8] that this fish grew best on the 30% protein diet when its digestible energy was sufficient in diets, pointing out that daily feed consumption was affected by dietary protein or cellulose levels.

In the previous study, we revealed that the optimum dietary protein level for *T. nilotica* varied with the kinds and levels of non-protein energy sources such as lipids and digestible carbohydrates in casein diets.[5] As a result, the optimum dietary protein level was estimated to be 30−40% when the casein diet contained sufficient energy sources (12−15% lipids and 30−40% digestible carbohydrates). The present study indicated that *T. nilotica* fingerlings grew optimally on diets containing about 35% protein in the casein-gelatin (3 : 1) diets regardless the digestible carbohydrate levels (30% and 40%) examined when diets contained 12% lipids. The present study also showed that the weight gain of *T. nilotica* improved with increasing dietary digestible energy (DE) levels (kcal/100 g), but the optimum DE levels varied with dietary digestible carbohydrate levels (Fig. 4). When the diets contained 30% digestible carbohydrate, the optimum DE level for *T. nilotica* was about 380 kcal/100 g as observed for casein diets previously.[5] Whereas, the optimum ratio of DE (kcal/kg) to protein (%), DE/P, was about 110 regardless the dietary digestible carbohydrate levels (Fig. 4) and slightly higher than those reported for other fish such as the brook trout, rainbow trout, channel catfish, carp, and yellow tail.[9,10] WANG *et al.*[11] have shown the optimum DE/P ratio for *T. nilotica* was 140−150, and also that an optimum dietary protein level was 25% at the feeding rate of 3.5% of body weight per day. Thus, the results of the present study did not agree with those of WANG *et al.*[11] However, the optimum dietary protein levels for fish may differ with the fish size, feeding rate, composition of non-protein energy sources, and other factors such as water temperature. Further work will be required to clarify the nutritional requirements of *T. nilotica* in relation to various factors in well designed experiments.

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