# Effect of Quality of Dietary Protein on Body Composition and Hepatopancreatic Enzyme Activities in Carp

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In order to investigate the effect of quality of dietary protein on hepatopancreatic enzyme activities and body component levels in carp *Cyprinus carpio*, the fish were fed a casein control diet and five diets containing 12.5% and 25% of gluten and 10%, 20%, and 30% of gelatin for 30 days. The growth rate and protein efficiency ratio of the fish fed diets containing 12.5% and 25% of gluten and 10% of gelatin were comparable to those of the fish fed the casein control diet, while the growth performance and apparent protein retention of the fish fed diets containing 20% and 30% of gelatin were markedly low, indicating insufficiency and unbalance of essential amino acid in both gelatin diets. Moreover, the hepatopancreatic glycogen content and serum urea level increased with increasing level of dietary gelatin. However, the activities of hepatopancreatic enzymes relating to glycolysis, gluconeogenesis, lipogenesis, and amino acid degradation were scarcely influenced by dietary treatments.

Key words: carp, dietary protein, carbohydrate metabolism, hepatic enzyme

In mammals, the quality of dietary protein affects several hepatic enzyme activities. For example, it was shown in rat that dietary gluten, gelatin, and soybean protein reduced more strongly hepatic lipogenic enzyme activities than fish protein and casein, [1,2] and that pyruvate kinase activity decreased with increasing level of dietary protein and its reduction was more remarkable in the animals fed the protein having higher biological value. [3] Therefore, there are some attempts to use enzyme activity for the evaluation of protein quality. On the other hand, Cowey *et al.* [4] compared the hepatic gluconeogenic enzyme activities in rainbow trout fed fish meal, casein, and gluten and described that those enzyme activities somewhat reflected the nutritional quality of those proteins. However, the relationship between protein quality and hepatic enzyme activity is still unclear in fish.

In recent years, many studies on alternative protein source for fish meal have been conducted very actively, [5] but little attention has been given to the effect of protein source on the metabolism in fish. In the present study, therefore, casein, gluten, and gelatin, which are similarly high in digestibility but different in nutritive value, were used as dietary protein source, and their effects on the hepatopancreatic enzyme activities and body component levels in carp were investigated.

## Materials and Methods

### Experimental Diets

Six isonitrogenous and isoenergetic purified diets were formulated (Table 1). In diet 1 (control diet), casein was used as a sole protein source. In diets 2 to 6, 12.5% and 25% of gluten and 10%, 20%, and 30% of gelatin were supplemented to the diets by replacing the corresponding level of casein in the control diet. All ingredients were mixed well with water and were made into pellets of 3 mm in diameter. All diets were stored in a freezer at −30 ℃. The contents of crude protein, crude sugar, and crude fat in the diets were *ca.* 42%, 34%, and 3%, respectively.

The essential amino acid (EAA) composition of the protein sources is shown in Table 2. Since whole egg protein has a high nutritive value for many fishes, its amino acid composition is also shown for reference. Casein was low in sulfur containing amino acids (methionine and cystine) and arginine compared with whole egg protein. On the other hand, gluten contained more sulfur containing amino acids than casein, and gelatin was rich in arginine, while both protein sources were poor in the other essential amino acids. Therefore, total EAA content in the diets decreased with increasing level of gluten and gelatin (Table 3), while the content and A / E ratio of sulfur containing amino acids and arginine were increased by dietary supplementation with gluten and gelatin.

### Fish and Feeding Methods

Yearling carp *Cyprinus carpio* purchased from a fish farmer in Nankoku City were reared on a commercial diet until used in the Freshwater Feeding Room, Kochi University. Then the fish were placed into 150 *l* fiber reinforced plastic aquaria containing aerated water. After one week of acclimation period, the fish averaging 41.2 g were divided into 6 groups of 17 fish each, and they were fed each test diet twice a day at the same daily feeding rate (1.61 ～ 1.71%). Unfiltered well water was supplied at a rate of approximately 2 *l*/min. The feeding trial was conducted for 30 days from May

**Table 1.** Formulation and proximate composition of experimental diets (%)

| Diet : | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Protein : | Casein | Gluten | | Gelatin | | |
| Level : | | 12.5% | 25% | 10% | 20% | 30% |
| Casein *[1] | 45.0 | 34.0 | 23.0 | 34.0 | 23.0 | 12.0 |
| Wheat gluten *[2] | | 12.5 | 25.0 | | | |
| Gelatin *[3] | | | | 10.0 | 20.0 | 30.0 |
| Carbohydrate mixture *[4] | 35.0 | 34.0 | 33.0 | 35.0 | 35.0 | 35.0 |
| Cellulose | 7.0 | 6.5 | 6.0 | 8.0 | 9.0 | 10.0 |
| Others *[5] | 13.0 | 13.0 | 13.0 | 13.0 | 13.0 | 13.0 |
| *Nutrient on dry matter basis* | | | | | | |
| Crude protein | 40.3 | 41.0 | 41.6 | 43.6 | 43.5 | 44.5 |
| Crude sugar | 33.7 | 34.3 | 34.7 | 33.6 | 34.2 | 34.7 |
| Crude fat | 3.1 | 3.8 | 4.6 | 3.0 | 3.1 | 2.7 |
| Energy (kcal/kg) *[6] | 3240 | 3350 | 3450 | 3370 | 3400 | 3430 |

*[1] Vitamin free casein, ICN Biochemicals, Ltd.
*[2] Wheat gluten, Wako Pure Chemical Industries, Ltd.
*[3] Bacto-gelatin, Difco Laboratories.
*[4] Dextrin: $\alpha$-starch=1:1.
*[5] Others (% in diet): pollack liver oil, 5; Halver's[20] vitamin mixture, 3; Ogino's[21] mineral mixture, 5.
*[6] Digestible energy (kcal/g): protein, 4.5; fat, 8.0; sugar, 3.5.

**Table 2.** Essential amino acid composition of casein, gluten, gelatin, and whole egg (% in protein)

| Protein : | Casein | Gluten | Gelatin | Whole egg |
|---|---|---|---|---|
| Threonine | 4.60 | 2.57 | 1.58 | 4.64 |
| Valine | 6.50 | 3.86 | 2.73 | 6.56 |
| Methionine+Cysteine | 3.92 | 4.64 | 1.06 | 6.08 |
| Isoleucine | 5.10 | 3.36 | 1.17 | 5.28 |
| Leucine | 9.88 | 6.80 | 0.97 | 8.48 |
| Phenylalanine+Tyrosine | 11.15 | 8.00 | 2.71 | 8.96 |
| Lysine | 8.40 | 1.65 | 3.64 | 7.04 |
| Histidine | 2.99 | 2.10 | 0.93 | 2.56 |
| Arginine | 3.85 | 3.42 | 7.80 | 6.40 |
| Tryptophan | 1.35 | 0.98 | 0.02 | 1.60 |
| Total | 57.74 | 37.38 | 22.61 | 57.60 |

25, 1993. Water temperature was in the range of 16.9 ~ 17.9 ℃.

### Analytical Methods

The fish were weighed every 10 days, and the weight gain and feed efficiency were calculated. At 15 to 17 h after the last feeding, five fish were sampled from each group, and the blood was taken from the caudal vein for analysis of serum components. Then the fish were sacrificed, and the hepatopancreas was removed, weighed, individually frozen in liquid nitrogen, and stored in a freezer at $-80$ ℃ until used for enzyme analysis.

The hepatopancreas was homogenized with 9 volumes of cold water by a Physcotron homogenizer (Nition Co., Ltd., NS-50) and centrifuged at 5,000 rpm for 10 min at 4 ℃. The supernatant was used to analyze the following enzymes: glucose-6-phosphatase (G6Pase, EC 3.1.3.9) ; fructose-1,6-diphosphatase (FDPase, EC 3.1.3.11) ; phosphofructokinase (PFK, EC 2.7.1.11) ; pyruvate kinase (PK, EC 2.7.1.40) ; glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49); phosphogluconate dehydrogenase (PGDH, EC 1.1.1.44) ; NADP-dependent malate dehydrogenase

(NADP-MDH, EC 1.1.1.40) ; NADP-dependent isocitrate dehydrogenase (NADP-ICDH, EC 1.1.1.42) ; aspartate aminotransferase (Asp-AT, EC 2.6.1.1) ; alanine aminotransferase (Ala-AT, EC 2.6.1.2) ; and arginase (EC 3. 5. 3. 1). The enzyme activities were assayed by the methods described previously.[6-9] Serum glucose, total protein, urea, free fatty acid, triglyceride, phospholipid, and cholesterol levels were determined with Wako kits (Wako Pure Chemical Industries, Ltd.), and serum free amino acid level was determined by the method of Goodwin.[10] The proximate composition of hepatopancreas and whole body was assayed by ordinary methods. The total sugar level in whole body and diets was assayed by the phenol-sulfuric acid method.[11] The hepatopancreatic glycogen level was determined by the methods of Carroll et al.[12] Statistical analysis of the serum component levels and enzyme activities was done by the Student's t-test.

### Results

#### Growth Performance and Body Composition

The weight gain and protein efficiency ratio (PER) of the fish fed diets 2, 3, and 4 were comparable to those of the fish fed the control diet (diet 1), while the fish fed diets containing higher level of gelatin (diets 5 and 6) showed low growth performance (Table 4).

In the hepatopancreas, the glycogen content markedly increased with increasing level of dietary gluten and gelatin, while conversely the protein content slightly decreased (Fig. 1). The whole body protein content was slightly high in the fish fed the gelatin diets (diets 4, 5, and 6). Based on the results of feed intake, weight gain, and the proximate analyses of whole body and diets, the apparent retentions of protein and energy were calculated (Table 4). The protein and energy retentions were similarly high in the fish fed diets 1, 2, 3, and 4, while they decreased in the fish fed diets 5 and 6.

**Table 3.** Essential amino acid composition of experimental diets (% in protein)

| Diet : | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Protein : | Casein | Gluten | | Gelatin | | |
| Level : | | 12.5% | 25% | 10% | 20% | 30% |
| Threonine | 4.60( 80)* | 4.10( 78) | 3.60( 75) | 3.86( 79) | 3.12( 77) | 2.38( 75) |
| Valine | 6.50(113) | 5.85(111) | 5.21(109) | 5.58(114) | 4.65(115) | 3.73(117) |
| Methionine+Cysteine | 3.92( 68) | 4.10( 78) | 4.27( 89) | 3.22( 66) | 2.52( 62) | 1.82( 57) |
| Isoleucine | 5.10( 88) | 4.67( 89) | 4.25( 89) | 4.14( 84) | 3.18( 78) | 2.21( 69) |
| Leucine | 9.88(171) | 9.12(173) | 8.37(175) | 7.70(157) | 5.52(136) | 3.34(105) |
| Phenylalanine+Tyrosine | 11.15(193) | 10.37(197) | 9.60(201) | 9.08(185) | 7.02(173) | 4.96(155) |
| Lysine | 8.40(146) | 6.74(128) | 5.09(107) | 7.23(147) | 6.07(150) | 4.91(154) |
| Histidine | 2.99( 52) | 2.77( 53) | 2.55( 53) | 2.48( 51) | 1.98( 49) | 1.48( 46) |
| Arginine | 3.85( 67) | 3.74( 71) | 3.64( 76) | 4.81( 98) | 5.78(143) | 6.74(211) |
| Tryptophan | 1.35( 23) | 1.26( 24) | 1.17( 25) | 1.03( 21) | 0.70( 17) | 0.37( 12) |
| Total | 57.74 | 52.72 | 47.75 | 49.13 | 40.54 | 31.94 |

* Numbers in parentheses indicate A/E ratio (1000×amino acid/total essential amino acid).

**Table 4.** Growth performance and apparent retentions of the fish fed test diets for 30 days

| Diet : | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Protein : | Casein | Gluten | | Gelatin | | |
| Level : | | 12.5% | 25% | 10% | 20% | 30% |
| Daily feeding rate (%) [*1] | 1.64 | 1.64 | 1.64 | 1.61 | 1.65 | 1.71 |
| Initial body weight (g) | 41.1 | 41.1 | 41.1 | 41.3 | 40.5 | 41.1 |
| Final body weight (g) | 66.8 | 69.5 | 68.1 | 69.3 | 62.1 | 57.0 |
| Average weight gain (g) | 25.7 | 28.4 | 27.0 | 28.0 | 21.6 | 15.9 |
| Feed efficiency (%) [*2] | 97.3 | 104 | 101 | 105 | 85.2 | 63.4 |
| Protein efficiency ratio [*3] | 2.42 | 2.55 | 2.42 | 2.41 | 1.96 | 1.42 |
| Apparent protein retention (%) [*4] | 35.9 | 33.7 | 31.6 | 36.0 | 30.1 | 24.3 |
| Apparent energy retention (%) [*4] | 57.5 | 54.0 | 54.4 | 54.6 | 46.7 | 38.5 |

[*1] 100×Dry diet fed (g)/100g body weight/day.
[*2] 100×Weight gain (g)/dry diet fed (g).
[*3] Weight gain (g)/protein fed (g).
[*4] 100×Nutrient deposited (g)/nutrient fed (g).
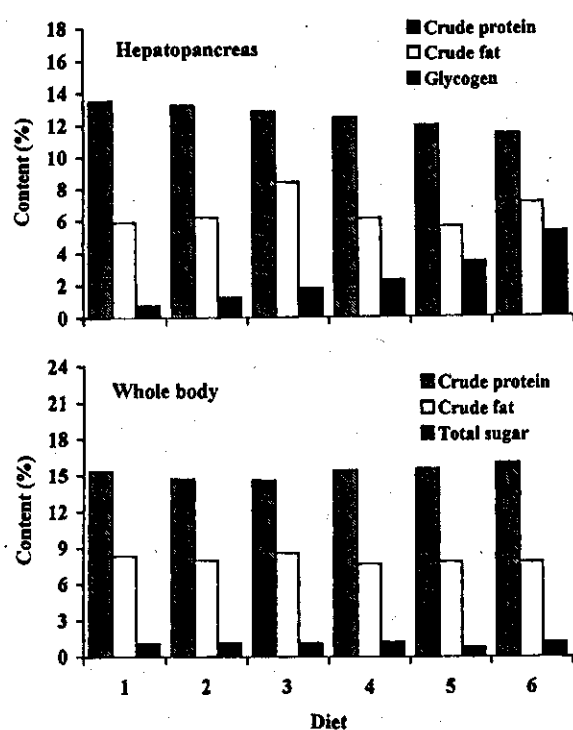


**Fig. 1.** Proximate composition of the fish fed test diets for 30 days. Samples were pooled for 6 (hepatopancreas) and 3 (whole body) fish.

*Hepatopancreatic Enzymes and Serum Components*

The results of hepatopancreatic enzyme activities and serum component levels were summarized in Table 5. Of the enzyme activities assayed, Ala-AT activity showed a tendency to decrease with increasing level of dietary gluten or gelatin. However, there was no remarkable difference in the other enzyme activities. The serum urea level significantly increased with increasing level of dietary gelatin. On the other hand, triglyceride, phospholipid, and cholesterol levels were slightly low in the fish fed diets 5 and 6. The other serum component levels did not show a consistent response to dietary treatments.

**Discussion**

Generally, casein has a high nutritive value, but sulfur containing amino acids and arginine are slightly insufficient. Conversely, gluten contains more sulfur containing amino acids than casein, and gelatin is rich in arginine. Therefore, the shortage of sulfur containing amino acids and arginine in casein can be compensated for by gluten and gelatin. In this experiment, total EAA content of diets containing 12.5% and 25% of gluten and 10% of gelatin was lower than that of the control diet, while the growth rate and PER of the fish fed these three diets were comparable to those of the fish fed the control diet. This result

**Table 5.** Hepatopancreatic enzyme activities and serum component levels of the fish fed test diets for 30 days

| Diet : | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Protein : | Casein | Gluten | | Gelatin | | |
| Level : | | 12.5% | 25% | 10% | 20% | 30% |
| *Hepatopancreatic enzyme* | | | | | | |
| G6PDH | 209±18[*1] | 225±48 | 212±42 | 207±32 | 197±41 | 190±46 |
| PGDH | 62.5±7.1 | 63.0±11.9 | 57.0±12.7 | 64.2±8.9 | 57.7±13.9 | 56.7±11.8 |
| NADP-MDH | 161±23 | 159±13 | 149±23 | 132±29 | 157±28 | 151±18 |
| NADP-ICDH | 120±21 | 129±13 | 115±11 | 114±11 | 115±14 | 104±13 |
| PFK | 43.1±6.3 | 46.7±8.0 | 43.6±6.1 | 53.2±7.6 | 47.1±7.8 | 50.1±4.3 |
| PK | 54.5±23.9 | 47.4±5.5 | 45.2±1.1 | 51.6±14.4 | 52.6±8.7 | 63.7±22.8 |
| G6Pase | 13.7±1.0 | 12.1±1.8 | 14.9±1.6 | 11.8±2.3 | 12.4±3.6 | 13.2±2.2 |
| FDPase | 8.73±1.36 | 9.63±1.62 | 9.53±1.04 | 10.8±0.9†[*2] | 8.23±1.54 | 8.08±2.52 |
| Asp-AT | 73.1±9.0 | 93.8±18.8 | 82.0±12.7 | 83.9±9.3 | 79.0±7.0 | 72.6±14.3 |
| Ala-AT | 11.9±1.1 | 10.6±1.4 | 9.82±0.87‡ | 10.0±3.2 | 8.34±1.93‡ | 8.30±2.01‡ |
| Arginase | 7.93±1.55 | 10.2±2.4 | 9.57±0.71 | 8.98±1.41 | 8.28±1.50 | 7.72±1.30 |
| *Serum component* | | | | | | |
| Glucose (mg/100m*l* ) | 44.1±8.5 | 44.6±8.3 | 33.9±6.9 | 31.8±7.1† | 42.2±12.1 | 41.6±11.8 |
| Triglyceride (mg/100m*l* ) | 434±139 | 352±45 | 436±102 | 606±221 | 294±101 | 333±60 |
| Cholesterol (mg/100m*l* ) | 206±37 | 184±20 | 210±23 | 224±66 | 172±27 | 185±10 |
| Phospholipid (mg/100m*l* ) | 609±81 | 620±49 | 731±77† | 700±111 | 552±97 | 588±63 |
| Free fatty acid (mEq/1000m*l* ) | 0.641±0.210 | 0.413±0.097 | 0.476±0.071 | 0.731±0.146 | 0.587±0.070 | 0.536±0.128 |
| Free amino acid (mg-N/100m*l* ) | 14.9±2.3 | 14.8±3.9 | 17.0±2.6 | 11.8±1.9† | 11.6±4.3 | 14.7±1.8 |
| Total protein (g/100m*l* ) | 2.56±0.33 | 2.49±0.16 | 2.48±0.23 | 2.31±0.29 | 2.36±0.22 | 2.38±0.16 |
| Urea (mg-N/100m*l* ) | 5.32±0.90 | 4.33±0.46 | 3.92±0.82† | 4.58±0.56 | 6.71±2.33 | 10.3±2.3‡ |

[*1] Enzyme activities are expressed as $\mu$ mol of substrate or coenzyme converted per min per hepatopancreas per 100g body weight. Values are means±SD for 5 fish.
[*2] Significant differences between the fish fed diet 1 and those fed the other diets are indicated by: † $P<0.05$; ‡ $P<0.01$.

suggests that the amino acid balance of dietary protein was improved by combinational use of casein and gluten or casein and gelatin. On the other hand, the fish fed diets containing 20% and 30% of gelatin showed low growth performance, indicating the insufficiency and unbalance of EAA in both gelatin diets.

Ingested protein enter the amino acid pool in body and are used for the synthesis of body protein. When large amounts of amino acids enter the pool, excess amino acids are deaminated and then the carbon skeletons are oxidized for energy production or used as the precursor for lipogenesis and gluconeogenesis. Likewise, if the biological value of dietary protein is low, more amino acids can be available for energy production or the synthesis of lipid and glycogen. In this study, the fish fed diets containing 20% and 30% of gelatin showed low protein retention and high hepatopancreatic glycogen content, while the analytical results of the hepatopancreatic enzyme activities did not show any evidence of the activation of amino acid degradation, lipogenesis, and gluconeogenesis. Fish naturally have high dietary requirement for protein and preferentially oxidize amino acids as energy source.[13,14] Therefore, the metabolic difference between the fish fed high quality protein and those fed low quality protein may be not very great. For this reason, the hepatopancreatic enzymes presumably failed to respond to the quality of dietary protein.

Amino acid degradation is regulated by tissue amino acid concentrations as well as enzyme induction.[15,16] Since amino acid degrading enzymes are not normally

saturated with substrate under physiological conditions, the enzymes are automatically activated by the increased concentrations of tissue amino acids after feeding. In rainbow trout, the dietary supplementation with lysine, tryptophan, and arginine raised the oxidation rates of ^{14}C-labeled lysine, tryptophan, and arginine to [14]$CO_2$ without affecting the hepatic lysine-$\alpha$-ketoglutarate reductase, tryptophan pyrrolase, and arginase activities.[16] This observation suggests that amino acid degradation was regulated by tissue amino acid concentrations. In the present study, arginase activity were hardly affected by dietary supplementation with gelatin, while the serum urea level was markedly increased. In teleost fish, urea is mainly formed *via* the breakdown pathway of purine base, while a portion of urea can arise from the hydrolysis of arginine by arginase.[17] Therefore, there is a possibility that a high concentration of tissue arginine due to the ingestion of gelatin diets activated arginase *in vivo*, resulting in increased level of serum urea. On the other hand, Shimeno *et al.*[18] reported that the activities of arginase, Asp-AT, and Ala-AT in the hepatopancreas of carp were increased by an increase in dietary protein level. In rat,[19] tryptophan and methionine have great effects on the inductions of hepatic arginase and threonine dehydratase. Hence, the amino acid degrading enzymes in the hepatopancreas of carp may be also induced by some nonsubstrate functional amino acids.

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