PURIFIED DIET DEVELOPMENT AND RE−EVALUATION OF THE DIETARY PROTEIN REQUIREMENT OF FINGERLING RAINBOW TROUT (Onconhynchus mykiss)*

Kim, K. I., Terrence B. Kayes and Clyde H. Amundson

ABSTRACT

Two experiments were carried out to : 1) develop a purified diet, and 2) exanine the protein requirement of fingerling rainbow trout. Four replicate tanks (50 fish each) of trout were assigned to a commercial salmon diet of a purified diet containing 30, 35, or 45% protein ; and three replicate tanks(40 fish each) to a diet containing 10, 15, 20, 25 or 35% protein(not counting crystalline dispensable amino acids which replaced casein protein to vary the protein level). Trout were fed three times a day for six weeks, and weight gain and feed/gain ratio were monitored.

No significant differences were found in weight gain or feed/gain ratio among 10. 5−g fish fed diets containing 30, 35, 40 and 45% protein. Fish fed these diets gained abjout 80% as much weight as fish fed a commercial salmon diet which produced gain of 19.5 g/fish/6 weeks. Weight gain increased with the increasing levels of casein up to 25% and the breakpoint was found at 24% intact protein, indicating that the levels of indispensable amino acids (IDAA) supplied by the 24% intact protein was sufficient to meet the requirements of trout for IDAA. Results suggested that energy sources used for trout diets play an important role in the determination of the protein requirements, and that the conventionally established protein requirement (40%) includes protein (24%) required to meet energy needs.

INTRODUCTION

Dietary protein is one of the major determinants of fish growth. Many studies have been done to determine protein requirements of various fish species(wilson, 1989). Most of the studies were carried out by varying the level of dietary protein at the expense of carbohydrates such as dextrin (Delong et al., 1958 ; Cowey et al., 1972 ; Anderson et al., 1981), wheat middlings(Cho et al., 1976) and starch(Lee and Putnam, 1973). Such studies indicated that fish require a relatively

* 本 論文은 현재 "Agriculture"에 투고중임.

high level of dietary protein(35 to 55%) overlooking the poorly difined nature of carbohydrates as energy substitutes for protein. Dietary proteins generally have higher matabolizable energy(ME) values than carbohydrates and thus, in many protein requirement studies, fish were probably offered higher metabolizable energy with the higher protein diets compared with the low protein diets.

The protein requirement of a given fish species may be dictated by dietary protein-energy ratio, protein quality(availability and balance of amino acids), energy sources and environments(temperature and salinity) (Millikin, 1982). Therefore, it is critical to the determination of protein requirements to standardize dietary ingredients, especially enery sources. Earlier studies at our laboratory(Sminth, 1986) showed that dietary protein (casein) could be replaced with alanine or a dispensable amino acid mixture. but could not be replaced with dextrin, on an eqal ME basis.

The studies reported here are : 1) to develop a purified(test) diet that promotes normal growth and 2) to examine the protein requirement of rainbow trout(Oncorhynchus mykiss) using a dispensable amino acid mixture to replace casein protein.

MATERIALS AND METHODS

Animals and feeding

Kamloop strain(experiment 1) and Shasta strain(experiment 2) rainbow trout were used. Prior to allotment of fish into experiments, fish were held in 750-1 round fiberglass flow-through holding tanks at 11℃~15℃. Four replicate groups, each containing 50 fish(experiment 1) or three replicate groups of 40 fish(experiment 2) of similar size were randomly selected from holding tanks and assigned to experimental tanks.

The experimental tanks(round, fiberglass, surfaced internally with a non-reflective green polyester gel coat) were arranged in an environment-controlled room. Tanks used for experiments contained 92ℓ of water(63.5cm height, 76.5 cm diameter, 20cm stand pipe). Municipal well water supplied to the tanks was carbon-filtered and water temperature was adjusted to 15±0.5℃ by mixing cold and hot water with thermostatically controlled valves. Environmental conditions in fish tanks were : pH 8.2, water flow 2 to 4 ℓ/minute, aeration rate 20 to 30 ℓ/minute at 15 to 30 mm Hg pressure above atmosphere, disolved oxygen near saturation(8 mg/ℓ), dissolved ammonia nitrogen not greater than 0.2 mg/ℓ.

Tanks were covered with 1.3cm(bar measure) knotless nylon netting to prevent fish from escaping. Light was supplied from incandescent bulbs and the light intensity was about 0.28Lux at water surface. An electeronic timer and rheostat systems provided a photoperiod(16-h light/8-h dark) and 30-minute simulated sunrises and sunsets.

Fish were acclimated to experimental tanks for at least one week while being fed Silver Cup salmon diet. Sick of dead fish were replaced during the acclimation period but not after experiments were begun. fish were fed roughly to apparent satiation at 08 : 00, 12 : 00 and 16 : 00 during the acclimation and experimental periods.

Diets for experiment 1, which was done to evaluate purified diets, were formulated to contain 30, 35, 40 or 45% protein. The protein was supplied by 2% gelatin and varying levels of casien supplemented with arginine and methionine to make the levels equal to those in whole egg protein(Table Ⅰ). In this experiment Silver Cup salmon diet containing 50% protein was used as a reference diet. Diets for experiment 2, which was done to determine a dietary level of protein that is sufficient to meet the requirements for indispensable amino acids, contained 10, 15, 20, 25 or 35% protein. The protein was supplied by 2% gelatin and varying levels of casein supplemented with arginine and methionine, and the protein level was varied at the expense of dispensable amino acid mixture(Table II).

The air-dry ingredients were mixed, and fish oil plus α-tocopherol was added. This mixture was then made into a stiff dough by blending with 50℃ water, which contained NaOH when necessary to give a final dietary pH of about 6.6(pH of the casein-based diet). The dough was extrued with a Hobart noodle maker(Hobart model K45SS, Troy, Ohio, USA) and dried in a forced-air feed dryer(23℃). The dried noodles were broken into pellets using a food processor. The size of pellets was approximately 2mm in diameter and 5~10mm in length. The final moisture content of dry pellets was approximately 10%. The diets were prepared weekly and stored at 4℃ until used.

The fish in each tank were weighed as a group at the start of experiments(mean initial body weight± SEM, 10.5± 0.1 g/fish for 20 tanks in experiment 1, and 9.7± 0.1 g/fish for 15 tanks in experiment 2) and at two-week intervals there after for six weeks. Feed consumption was recorded and feed/gain ratio calculated for each two-week period and for the entire six-week period.

Carcass analysis

At the start of experiment 2, ten fish were randomly selected from a reserve tank kept under the same conditions as the experimental tanks and used to determine initial carcass composition. Final carcass composition was also determined with ten fish randomly selected from each experimental tank at the end of the experiment. The fish were weighed before and after evisceration. The carsasses were blended, dried and ground. Moisture content was determined by drying at 80℃ for 24 hours. Total organic nitrogen content was measured by using a semi-micro Kjeldahl procedure for digestion(AOAC, 1984), and the phenol-hypochlorite reaction for colorimeric determination of ammonium(Weatherburn, 1967). Total lipid content was measured using the method reported by Bligh and Dyer(1959) and ash content was determined by using the AOAC(1984) procedure. Nitrogen retention was calculated as follows ; 100(g N in carcass at the end of six weeks-g N in carcass at the start)/g N intake.

Statistical analysis

Data were subjected to analysis of variance. When differences were significant at less than 50%, the Newman-Keuls test(Snedecor and Cochran, 1980) was used to compare mean values of individual treatments. In experiment 2, a broken-line model(Robbins at al., 1977) was used to establish the protein requirement, assuming that the breakpoint represents the requirement, and that weight gains for the dietary protein levels at and greater than the requirement are estimates of the same respones.

RESULTS

Weight gain, specific growth rate and feed/gain ratio of fish fed a reference diet or purified diets are presented in Table III. The fish fed the reference diet gained more weight ($p<0.05$) than those fed purified diets(19.5 vs 15.9g). However, feed/gain ratio was not significantly ($p>0.05$) different between the reference and purified diets.

No significant differences were detected in weight gain or feed/gain ratio among trout fed the purified diets containing graded levels of protein from 30 to 45%, although feed/gain ratio appeared to decrease with increasing protein levels. Results of experiment 1 showed that the protein requirement of rainbow trout was not more than 30% when the protein was supplied by casein supplemented with arginine and methionine.

Weight gain increased linearly($r^2 = 0.998$) with protein levels up to 25% and breakpoint was found at 24% (table Ⅳ, Fig. 1), indicating that the dietary protein(24%) from casein and gelatin supplemented with arginine and methionine was sufficient to meet all the indispensable amino acids required for optimum growth. Mortality was high in experiment 2 for unknown reasons, but it was not related to diet quality.

Feed/gain ratio decreased with increasing levels of protein, but the difference between fish fed the 25 and 35% protein diets was not significant($p > 0.05$). although fish fed the diet containing 25% protein tended to consume more than those fed the diet containing either 20 or 35% protein (TableⅣ). Nitrogen retention of trout fed the 10 % protein diet was significantly($p < 0.05$) lower than that of the others(Table Ⅳ).

Carcass composition was not markedly influenced by the dietary protein level except for trout fed the 10% protein diet, which had relatively lower dry matter and lipid contents, and higher protein and ash contents(table Ⅴ).

DISCUSSION

Growth and feed utilization of animals fed purified diets are normally inferior to those of animals fed practical diets(Satia, 1974 ; Kim et al., 1983). Nonetheless, the use of purified diets is inevitable for the studies of nutrient requirements. Requirements for protein or amino acids by fishes as well as other amimal species have been estimated using purified diets : e. g., rainbow trout(Satia, 1974 ; Pfeffer et al., 1980), chinook salmon(Halver et al., 1957), channel catfish(Wilson et al., 1977), carp(Aoe et al., 1970), pigs(Judley et al., 1962 ; Kim et al., 1983a), rats(Rogers and Harper, 1965) and chicks(Baker, 1977). In these studies, milk protein and crystalline amino acids were most frequently used as protein sources. In our studies, casein was a major protein source and the level of gelatin was minimized (2% of diets) because its nutritional values for rainbow trout had been questioned when compared with glucose, sucrose or gelatinized starch(Pieper and Preffer, 1980).

Some fish nutritionists have been using specific growth rates in an attempt to correct growth rates of fish for initial body weight because fish tend to gain their weight exponentially with time in their early stages of life. Results from different laboratories show large variations in specific growth rates. The rates vary from 1.2 (Hughes et al., 1981) to 2.75(Satia, 1974), or 3.78(Ogino and Nanri, 1980). The specific growth rate obtained with 3.5-g rainbow trout by Satia(1974) seems extraordinarily high, after the extreme variation in the water temperature(16~27℃) was considered. A high specific growth rate observed by Ogino and Nanri(1980) is also difficult to verify because they fed the fish for only 19 days.

As shown in Table III, our purified diet appeared to promote relatively good specific growth rate(2.07~2.23) and feed utiliztion(feed/gain ratio, 1.43~1.27), although it is somewhat inferior to the specific growth rate(2.48) and feed utiliztion(feed/gain ratio, 1.32) of fish fed the Silver Cup salmon diet. Therefore, use of the purified diet for the determination of the protein or amino acid requirements is considered an acceptable compromise. The specific growth rate observed in experiment 1 was inferior to that obtained in the other experiments(Tables III, IV) maily because of the strain(Kamloop) used. This strain seemed to be more aggressive and cannibalistic than other strains, especially when fish were confined in experimental tanks and dissatisfied with diet.

Results of experiment 2(Table IV, Fig. 1) suggest that the protein level(40%) recommended by NRC(1981) includes dietary protein required to meet the requirements for the indispensable amino acids(24%) and that required for meeting energy needs(16%). This suggestion is well supported by the estimated indispensable amino acid requirements of rainbow trout : arginine, 1.4% of the diet(Kim et al., 1983b) ; lysine, 1.3%(Kim and Kayes, 1982) ; sulfur-containing amino acids, 0.8%(Kim et al., 1984) ; and tryptophan, 0.2%(Kim et al., 1987) as compared with the amino acid levels in the 25% protein diet used in the present experiment ; 1.6, 1.9, 1.0, and 0.27 % of the diet, respectively.

Earlier studies at our laboratory(Smith, 1986) showed that a diet containing 25% protein(from 25% casein and 2% gelatin) and 10% alanine or 10% dispensable amino acid mixture promoted growth of rainbow trout as well as a diet containing 35% protein(from 36% casein and 2% gelatin). In that study, fish(initial body weight, 8.6g) fed the control diet containing 35% protein, and diets containing 25% protein and 10% dispensable amino acid mixture, 10% alanine or 10% (above the basal level) dextrin gained 28.5, 28.4, 27.2 and 25.5g over a 6-week feeding period, respectively. This result again suggests that the 25 % protein from 25% casein and 2% gelatin in the diet was enough to meet the requirements of rainbow trout for the indispensable amino acids, and further suggests that the protein requirement of rainbow is not more than 25% when appropriate energy sources that have ME values equivalent to protein are used to substitute for protein.

Most published data regarding the protein requirement of rainbow trout(Zeitoun et al., 1973 ; Lee and Putnam, 1973 ; Satia, 1974 ; Austreng and Refstie, 1979) have shown that rainbow trout require more than 40% protein in their diets. This value was obtained by using various carbohydrates as substitues for protein. Considering the confounding effect of energy sources on the protein requirement, one needs to be careful in interpretation of data on the protein requirements of fish and other carnivores. Data on the protein requirement of kittens are variable as reported to be 25~30% by Miller and Allison(1958), 37~43 % by Dickinson and Scott(1956) and 31~36% by Janson et al.,(1975). These differences in the estimated protein requirements among the studies may be due to the energy content of the diets (MecDonald et al., 1984).

Collectively, results of our studies indicate that energy sources in fish diets influence the protein requirement of fish for optimum growth, and the metabolic partition of the dietary protein between use for protein synthesis and for energy supply. Therefore, the energy value of a diet must be defined in terms of its individual components before any conclusions about the protein requirement of a particular species can be drawn.

Since alanine or a dispensable amino acid mixture were able to substitute for protein in trout diets on an equal ME basis, these materials can be used as a reference(standard) for other dietary energy sources. We propose that alanine equivalents of fish feed ingedients as an alternative to ME values be established by comparing the growth of fish fed an energy source of interest with the growth of fish fed alanine. This alanine-equivalent method would be more useful than the conventional ME measurements because the ME value of a given dietary ingredient can vary with many factors, such as species and size of fish, environments and other dietary ingredients.

ACKNOWLEDGEMENTS

The authors thank Penny Swanson, Bill Manci and Tom Kuczynski for their technical help. This work was supported in part by the University of Wisconsin–Madison College of Agricultural and Life Sciences and by the University of Wisconsin Sea Grant College Program, National Oceanic and Atmospheric Administration, U. S. Department of Commerce, and from the State of Wisconsin. Federal grant NA80AA–D00086, projects R/AQ–8 and R/AQ–9.

REFERENCES

Anderson, R. J., Kienholz, E. W. and Flickinger, S A., 1981. Protein requirements of smallmouth bass and largemouth bass. J. Nutr., 111 : 1085–1097.

AOAC(Association of Official Aanlytical Chemists), 1984. Official Methods of Analysis, 14th edition, W. Horwits [Editor], Washington, D. C., pp. 125~126.

Aoe, H., Matsudo, I., Saito, T., Toyoda, T. and Kitamura, K., 1970. Nutrition of protein in young carp. 1. Nutritive value of free amino acids. Bull. Jpn. Soc. Sci. Fish., 36 : 407~413

Austreng, E. and Refsting, T., 1979. Effect of varying dietary protein levels in different families of rainbow trout. Aquaculture, 18 : 145~156.

Baker, D. H., 1977. Amino acid nutrition of the chick. In : H. H. Draper [Editor], advances in Nutritional Research. Plenum Press, New York. pp. 299~335.

Bligh, E. C and Dyer, W. J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. POhysiol., 37 : 913~917.

Cho, C. Y., Slinger, S. J. and Bayley, H. S. 1976. Influence of level and type of dietary protein, and of level of feeding of feed utilization by rainbow trout. J. Nutr., 106 : 1547~1556.

Cowey, C. B., Pope, J. A., Adron, J. A. and Blair A., 1972. Studies on the nurtition of marine flatfish. The protein requirement of plaice (Pleuronectes platessa). Br. J. Nutr. 28 : 447~456)

Delong., D. C., Halver, J. E. and Mertz, E. T., 1958. Nutrition of salmonid fishes. IV. Protein requirements of chinook salmon at two different water temperatures. J. Nutr., 65 : 589~599.

Dickinson, C. D. and Scott, P. O., 1956. Nutrition of the cat. 2. protein requiements for growth of weaning kittens and young cats maintained on a mixed diet. Br. J. Nutr., 10 : 311~316.

Dudley, W. A., Becker, D. E., Jensen, A. H., Terrill, S. W. and Norton, H. W., 1962. Crystalline amino acid mixtures as the sole soure of nitrogen for the baby pig. J. Animal Sci., 21 : 639~642.

Hughes, S. G., Rumsey, G. L. and Nickum, J. G., 1984. Riboflavin requirement of fingerling rainbow trout. Prog. Fish–Cult., 43 : 167~171.

Jansen, G. J., Deuth, M. A., Ward, G. M. and Johnson, D. E., 1975. Protein quality studies in growing kittens. Nutr. Rep. Int., 11 : 525~536.

Kim, K. I. and Kayes T. B., 1982. Test diet development and lysine requirement of rainbow trout. Fed. Proc. 41 : 716(Abst).

Kim, K. I., McMillan, I. and Bayley, H. S., 1983a. Determination of amino acid requirement of young pigs using an indicator amino acid. Br. J. Nutr., 50 : 369~382.

Kim, K. I., Kayes, T. B. and Amundson, C. H., 1983b. Protein and arginine requirements of rainbow trout. Fed. Proc. 42 : 667(Abst).

Kim, K. I., Kayes, T. B. and Amundson, C. H., 1984. Requirements for sulfur-containing amino acids and utilization of D-methionine by rainbow trout. Fed. Proc., 43 : 856(Abst).

Kim, K. I., Kayes, T. B. and Amundson, C. H., 1987. Effects of dietary tryptophan levels on growth, feed/gain, carcass composition and liver glutamate dehydrogenase activity in rainbow trout(Salmo gairdeneri). Comp. Biochem. Physiol. 88B : 737~741.

Lee, D. J. and Putnam, G. E., 1973. The response of rainbow trout to varying protein/energy ratios in a test diet. J. Nutr., 102 : 916~922.

Macdonald, M. L., Rogers, Q. R. and Morris, J. G., 1984, Nutrition of the domestic cat, a mammalian carnivore. Ann. Rev. Nutr., 4 : 521~562.

Miller, S. A. and Allison, J. B., 1958. The dietary nitrogen requirements of the cat. J. Nutr., 64 : 493~501.

Millikin, M. R., 1982. Qualitative and quantitative nutrient requirements of fishes : A review. Fisheries Bull., 80 : 655~686.

NRC(National Research Council), 1981. Nutrient requirements of coldwater fishes. Nutrient requuirements of domestic animals series. National Academy of Science, Washington, D. C.

Ogino, C. and Nanri, H., 1980. Relationship between the nutritive value of dietary proteins for rainbow trout and essential amino acid compositions. Bull. Jpn. Soc. Sci. Fish., 46 : 109~112.

Pfeffer, V. E., Petrasch, R. and Eckhardt, O., 1980. Studies on possible sources of protein and fat in purified diets for rainbow trout. Zeit. Tierphysiol. Tierernah. Futtermittelkunde, 43 : 254~263.

Pieper, A. and Pfeffer, E., 1980. Studies on the comparative efficiency of utilization of gross energy from some carbohydrates, proteins and fats by rainbow trout(Salmo gairdneri, R.). Aquaculture, 20 : 323~332.

Robbins, K. R., Baker, D. H. and Norton, H. W., 1977. Histidine status of the chick as measured by growth rate, plasma free histidine and breast muscle carnosine. J. Nutr., 107 : 2055~2061.

Rogers, Q. R. and Harper, A. E., 1965. Amino aicd diets and maximal growth in the rat. J. Nutr., 87 : 267~·273.

Satia, B. P., 1974. Quantitative protein requirements of rainbow trout. Prog. Fish-Cult., 36 : 80~85.

Smith, D. A., 1986. Studies on the protein requirement of rainbow trout(Salmo gairdneri). Ph. D thesis, University of Wisconsin, Madison.

Snedecor, G. W. and Cochran, W. C., 1980. Statistical methods (8th ed.). The Iowa State University Press, Ames, Iowa, pp. 215~237.

Weatherburn, M. W., 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem., 39 : 971~974.

Wilson, R. P., Harding, D. E. and Garling, D. E., 1977. Effect of dietary pH on amino acid utilization and the lysine requirement of fingerling channel catfish. J. Nutr., 106 : 166~170.

Wilson, R. P., 1989. Amino acids and proteins. In : J. E. Halver [Editor], Fish Nutrition, 2nd ed., Academic Press, New york, pp. 111~151.

Zeitoun, I. H., Halver, J. E. Ullrey, D. E. and Tack, P. I., 1973. Influence of salinity on protein requirements of rainbow trout(Salmo gairdneri) fingerlings. J. Fish. Res. Bd. Can., 30 : 1867~1873.

TABLE I

Dietary Ingredients (% on a air-dry basis) - experiment 1

Diet [1]	1	2	3	4
Casein [2, 3]	30.67	36.11	41.54	46.98
Gelatin (89% CP) [2]	2.00	2.00	2.00	2.00
Dextrin, white [2]	31.40	25.78	20.18	14.56
Dextrose [2]	5.00	5.00	5.00	5.00
α-cellulose [4]	8.20	8.20	8.20	8.20
Fish oil [5]	10.00	10.00	10.00	10.00
CM-cellulose [2, 6]	1.00	1.00	1.00	1.00
Mineral mixture [7]	5.06	5.06	5.06	5.06
$CaHPO_4$ [8]	2.94	2.94	2.94	2.94
Vitamine mixture [9]	2.00	2.00	2.00	2.00
Choline chloride [2]	0.70	0.70	0.70	0.70
DL-α-tocopherol [2, 10]	0.0455	0.0455	0.0455	0.0455
Arginine [11]	0.69	0.82	0.94	1.08
Methionine [11]	0.29	0.34	0.39	0.43
Crude Protein(%) [12]	30	35	40	45

1) Silver Cup salmon diet containing 50% CP(Murray Elevators, Murray, Utah, USA) was used as a reference diet.

2) United States Biochemical Corporation, Cleveland, Ohio (USA).

3) Vitamin-free, 92% CP.

4) Sigma Chemical Company, ST. Louis, Missouri (USA).

5) Pacific Salmo, a gift from Dr. R. O. Sinhuber, Oregon State University, Corvallis, Oregon (USA).

6) Carboxy methyl.

7) As mg/kg of dry diet : Nacl 5842, Ki 1.91, $CaCO_3$ 4010, K_2HPO_4 15466, K_2SO_4 12984, Na_2HPO_4 2320, MgO 4774, $FeSO_4 7H_2O$ 1066, $MnCO_3$ 797, $CuCO_3Cu(OH)_2$ 64.9, $ZnCO_3$ 154.7, NaF 3.82, $CoCl_2 6H_2O$ 95.4, citric acid 3020.

8) Fisher Scientific Company, Fair Lawn, New Jersey (USA).

9) As mg/kg of dry diet : vitamin A acetate(500 IU/mg) 50, vitamin D3 (1000 IU/mg) 4, menadione 16, thiamine HCl 64, riboflavin 144, D-Ca-pantothenate 288, d-biotin 1.6, folic acid 19.2, vitamin B12(0.1%) 160, niacin 512, pyridoxine HCl 48, L-ascorbic acid 1200, myo-inositol 2500, p-amino-benzoic acid 400, diatomaceous earth 14139. Except for the latter, which came from Sigma Chemical Co., all the above ingredients were from United States Biochemical Corporation.

10) 1.1 IU/mg in acetate form.

11) Added to level proportional to that in whole egg.

12) Calculated(Analyzed values were similar to the calculated values with differences less than ±1%)

TABLE II

Dietary ingredients (% on a air-dry basis)-experiment 2

Diet [1]	1	2	3	4	5
Casein [1, 2]	8.92	14.36	19.80	25.22	36.11
L-DAA [3]	25.00	20.00	15.00	10.00	-
Dextrin, white [1]	28.83	28.20	27.58	26.96	25.71
Gelatin(89% CP) [1]	2.00	2.00	2.00	2.00	2.00
Dextrose [1]	5.00	5.00	5.00	5.00	5.00
α-cellulose [4]	8.20	8.20	8.20	8.20	8.20
Fish oil [5]	10.00	10.00	10.00	10.00	10.00
CM-cellulose [4, 6]	1.00	1.00	1.00	1.00	1.00
Mineral mixture [7]	5.06	5.06	5.06	5.06	5.06
$CaHPO_4$ [8]	2.94	2.94	2.94	2.94	2.94
Vitamine mixture [9]	2.00	2.00	2.00	2.00	2.00
Choline chloride [2]	0.70	0.70	0.70	0.70	2.00
DL-α-tocopherol [2, 10]	0.0455	0.0455	0.0455	0.0455	0.0455
L-Arginine [11]	0.176	0.311	0.446	0.581	0.851
L-Methionine [11]	0.130	0.180	0.230	0.281	0.380
Crude Protein(%) [12]	10	15	20	25	35

1) United States Biochemical Corporation, Cleveland, Ohio (USA).
2) Vitamin-free, 92% CP.
3) Dispensable amino acid mixture(%) ; ala. 15.74, asp 28.77, gly 5.41, glu 27.56, pro 1.25, ser 21.27.
4) Sigma Chemical Company, ST. Louis, Missouri (USA).
5) Atlantic herring oil from Glenco Mills, Incorporated, Gelenco, Minnesota (USA).
6) Carboxy methyl.
7) See footnote 7 to Table I.
8) Fisher Scientific Company, Fair Lawn, New Jersey (USA).
9) See footnote 9 to Table I.
10) 1.1 IU/mg in acetate form.
11) Added to level proportional to that in whole egg.
12) Calculated excluding the nitrogen attributable to the added dispensable amino acid mixture.

TABLE III

Effects of commercial or purified diets on growth and feed/gain ratio of rainbow trout[1] – experiment[1]

Diet	Level of protein (%)	Weight gain (g/fish)	Specific growth rate[2]	Feed/gain (g/g)	Mortality (%)
Reference	50	19.5 ± 0.6[a, 3]	2.48 ± 0.05[a]	1.32 ± 0.04	0.0
Purified 1	30	14.6 ± 0.6[b]	2.07 ± 0.03[b]	1.43 ± 0.04	0.5
2	35	14.9 ± 0.5[b]	2.11 ± 0.06[b]	1.39 ± 0.04	0.5
3	40	15.7 ± 0.5[b]	2.23 ± 0.06[b]	1.28 ± 0.03	0.5
4	45	15.9 ± 0.8[b]	2.20 ± 0.07[b]	1.27 ± 0.07	1.5

1) Mean initial body weight ± SEM was 10.5 ± 0.1 g/fish for 20 tanks and values are means ± SEM of 4 replicate tanks over the six−week period.

2) (In final weight − In initial weight) 100/42 days.

3) Values sharing the same superscript in the same column are not significantly different at the 5% level when analyzed by the Newman−Keuls test.

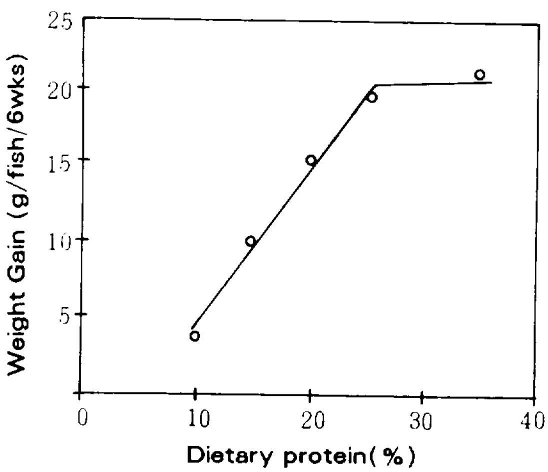

Fig. 1

Effect of dietary protein levels on weight gain in rainbow trout.

Each point indicates mean ± SEM of three replicate tanks. A broken−line model was used to find a breakpoint when the residual sum of square of the following regression is minimum.

$$Y = M(X-a) + b \ ; \quad X < a$$
$$Y = b \ ; \quad X > a$$

where ;

Y = weight gain

M = slope ± SEM (1.17 ± 0.11) of the ascending line

X = protein level

a = X at break point (24.01 ± 1.05)

b = Y at break point (20.00 ± 0.55)

TABLE Ⅳ

Effects of dietary protein levels growth, feed/gain ratio and nitrogen retention[1] − experiment[3]

Level of protein (%)	Weight gain (g/fish)	Specific growth rate [2]	Feed/gain (g/g)	Nitrogen retention [3]	Mortality (%)
10	3.5 ± 0.5 [a, 4]	0.72 ± 0.09 [a]	4.24 ± 0.78[a]	18.7 ± 6.1[a]	1.7
15	9.8 ± 0.7 [b]	1.67 ± 0.08 [b]	1.83 ± 0.11[b]	40.2 ± 3.2[b]	0.8
20	15.2 ± 1.0 [c]	2.25 ± 0.07 [c]	1.53 ± 0.06[c]	38.2 ± 2.2[b]	9.2
25	19.5 ± 0.3 [d]	2.65 ± 0.04 [d]	1.31 ± 0.02[d]	42.6 ± 0.7[b]	10.0
35	20.6 ± 1.2 [d]	2.68 ± 0.10 [d]	1.15 ± 0.03[d]	36.7 ± 0.9[b]	10.8

1) Mean initial body weight ± SEM was 9.7 ± 0.1 g/fish for 15 tanks and values are means ± SEM of three replicate tanks over the six-week period.
2) See footnote 2 to Table III.
3) 100(g N in carcass at the end of six weeks − g N in carcass ot the start)/g N intake over 6 weeks.
4) Values sharing the same superscript in the same column are not significantly different at the 5% level when analyzed by the Newman-Keuls test.

TABLE Ⅴ

Effects of dietary protein levels on carcass composition[1] − experiment[2]

Level of protein (%)	Carcass composition(%) [2]			
	Dry matter	Protein [2]	Lipid [3]	Ash [3]
10	23.7 ± 0.3 [a, 4]	60.3 ± 0.4	25.0 ± 1.3	10.8 ± 0.2[a]
15	25.7 ± 0.5 [b]	57.8 ± 0.7	28.9 ± 0.2	9.4 ± 0.0[b]
20	26.5 ± 0.4 [b]	57.3 ± 0.5	29.6 ± 0.8	9.0 ± 0.1[c]
25	27.7 ± 0.4 [c]	58.2 ± 1.0	29.3 ± 0.5	8.9 ± 0.1[c]
35	27.7 ± 0.3 [c]	58.7 ± 0.5	29.8 ± 0.5	8.8 ± 0.1[c]

1) Mean ± SEM of three replicate tanks.
2) Eviscerated carcass ; initial carcass composition was 23.4% dry matter, 71.4% protein, 19.3% fat and 10.6% ash.
3) On the dry matter basis.
4) Values sharing the same superscript in the same column are not significantly different at the 5% level when analyzed by the Newman-Keuls test.