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THE PROTEIN REQUIREMENT ON ENERGY BASIS FOR RAINBOW TROUT (*SALMO GAIRDNERI*).

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ABSTRACT

Hexane extracted herring meal was used as the sole protein source in diets to rainbow trout. The feed was further composed with capelin oil and partly dextrinized potato starch as energy supplements. Food intake, weight gain, feed efficiency, protein retention and productive protein value were calculated and compared relatively to the energy content from protein in the feed. The results indicated that the protein requirement for maximum growth of rainbow trout was about 0.37—0.41 parts of protein energy of the total dietary energy. At this level the fat content of the feed was about 200 g per kg dry weight, giving 0.40—0.42 part of the total energy.

INTRODUCTION

Many studies have been published on the protein requirement of salmonoid fishes, an extensive knowledge of which is necessary to ensure economic feeding in modern intensive fish farming. The protein supplement is normally given in weight for the dietary requirements (DELONG et al., 1958; CHO et al., 1976), without consideration of the energy components in the feed composition. The dietary energy level must be considered as fish, like other animals, eat to satisfy their energy requirement (ROZIN and MAYER, 1961; GERKING, 1971; LEE and PUTNAM, 1973; LOVELL, 1976).

In three feeding experiments with rainbow trout, the protein retention (body protein at the end of the trial, minus body protein at the beginning of the trial), productive protein value (protein retained over protein consumed), and feed efficiency were related to the dietary protein level on energy basis.

METHODS

DESCRIPTION OF DIETS

Hexane-extracted fish meal from a Norwegian factory,* was used as a stable protein source containing a minimum of fat. Fish oil from capelin and partly dextrinized potato starch ("dextrin") were used as energy

*«Norsamin».

supplies. The energy values of the components were calculated based on the following values: 16.38 kJ/g protein (3.9 kcal/g), 33.47 kJ/g fat (8.0 kcal/g) and 12.55 kJ/g "dextrin" (3.0 kcal/g). The dextrin energy level was kept at 20% of the total energy in the feed throughout the three experiments. The energy content from protein was therefore balanced by fish oil addition.

The dry products and the capelin oil were thoroughly mixed in a kitchen mixer and the necessary amount of water was slowly added. The moist diets were pelleted by means of a meat mincer to sizes suitable to the fish in each experiment. The diets were stored at — 18°C until used.

EXPERIMENTAL CONDITIONS

The fish was obtained at a local hatchery*, and belonged to the same lot, hatched in March 1974. They were kept on a commercial dry feed until transported to the laboratory.

The experiments were conducted in 8 aquaria each holding 225 liters. Each experiment was made up of 4 groups, run in duplicate. Freshwater with a temperature of 13.5°C (± 0.3°C), was given at a flow of 3 liters per minute. The aquaria were given 12 hour light, 12 hour darkness per day.

Diets in frozen, moist pellet form were offered 3 times a day, 5 days a week on a rigid schedule. The fishes were fed as long as they accepted the feed. The daily amounts consumed were calculated.

Body weights and feed intakes are given as averages. The fish sizes in the three experiments increased with time as the trials were conducted over a period of half a year. The number of fishes in each aquarium varied from 47 in experiment 1 to 20 in experiment 3, but the total biomass was approximately equal.

Analyses of variance and orthogenal comparisons were used to evaluate significance of differences among the means obtained.

ANALYTICAL PROCEDURES

Duplicate determinations of the content of dry matter, fat and protein were made on all feeds and on 10 fishes from the combined lot at the start of each experiment and on 5 or 10 fishes from each group at the end of the experiments.

Dry matter was determined by freeze-drying the samples. Five gram of dried material was defatted by extracting 3 times with 50 ml of ethyl ether, each time stirring for 5 minutes. Both lipid and lipid free fractions

*«Fisk og Forsøk», Matredal.

Table 1. Composition of the experimental diets. Weight and energy relations.

Expt. group	Fish meal				Fish oil				Dextrin			Total energy MJ/kg
	Weight g/kg	Protein g/kg	Energy MJ/kg	Energy PE/TE*	Weight g/kg	Extr.fat g/kg	Energy MJ/kg	Energy FE/TE	Weight g/kg	Energy MJ/kg	Energy DE/TE	
1	398	*322*	*5.28*	*.307*	260	*252*	*8.44*	*.491*	277	*3.48*	*.202*	17.20
2	491	*398*	*6.51*	*.408*	192	*186*	*6.23*	*.390*	257	*3.23*	*.202*	15.97
3	571	*463*	*7.58*	*.508*	134	*130*	*4.35*	*.291*	239	*3.00*	*.201*	14.93
4	641	*519*	*8.50*	*.606*	84	*81*	*2.73*	*.195*	223	*2.80*	*.200*	14.03
5	434	*352*	*5.76*	*.343*	236	*229*	*7.66*	*.456*	268	*3.36*	*.200*	16.78
6	457	*370*	*6.06*	*.368*	219	*212*	*7.11*	*.432*	262	*3.29*	*.200*	16.46
7	475	*385*	*6.30*	*.388*	206	*200*	*6.69*	*.412*	259	*3.25*	*.200*	16.24
8	500	*405*	*6.63*	*.417*	188	*182*	*6.10*	*.384*	252	*3.16*	*.199*	15.89
9	434	*352*	*5.76*	*.343*	236	*229*	*7.66*	*.456*	268	*3.36*	*.200*	16.78
10	457	*370*	*6.06*	*.368*	219	*212*	*7.11*	*.432*	262	*3.29*	*.200*	16.46
11	475	*385*	*6.30*	*.388*	206	*200*	*6.69*	*.412*	259	*3.25*	*.200*	16.24
12	500	*405*	*6.63*	*.417*	188	*182*	*6.10*	*.384*	252	*3.16*	*.199*	15.89

* PE/TE = Protein energy over total energy.
Further were added: 40 g/kg mineral premix, 15 g/kg vitamin premix and 5 g/kg carboxy-methyl-cellulose.

were dried to constant weight in an oven at 105°C. The protein determinations were carried out on lipid free material. Nitrogen contents were determined by a modified colorimetric method described by CROOK and SIMPSON (1971), giving crude protein by multiplying nitrogen values with 6.25.

RESULTS

Table 1 gives the composition of the experimental diets and table 2 the composition of the added minerals and vitamins. The protein content was found by analysis of the prepared diets to average 81 % of the fish meal and the extractable fat from the diets averaged 97 % of added oil.

The diets in experiment I, group 1 to 4 were composed to give 30, 40, 50 and 60 % of total energy from protein (PE/TE between 0.30 and 0.60). On the basis of the results of experiment I, the diets in experiments II and III, groups 5 to 12 were composed to give 34 to 42 % of total energy from protein. The carbohydrate energy content was kept at 20 % of the total in all groups. The remainder was added as fat and table 1 shows that the oil content of the diets varied between 8.5 and 26 weight percent. The total energy content of the diets varied between 14 and 17 MJ per kg, or approximately 3.500—4.000 kcal/kg.

Table 3 gives analyses of essential amino acids in the fish meal, and shows that all amino acids except arginine were found in concentrations well above those proposed by SHANKS et al. (1962) as requirements.

Table 4 gives values for dry matter, protein and fat in the analysed samples of fish after 4 weeks on the experimental diets. The fat content

Table 2. Composition of the vitamin premix and mineral premix used in the feed.

Thiamin-HCl	2.000 g/kg	$Ca_3(PO_4)_2$	360.00 g/kg
Riboflavin	2.000 ”	$CaCo_3$	95.00 ”
Pyridoxine	2.000 ”	KCl	250.00 ”
Niacin	10.000 ”	NaCl	50.00 ”
d-Ca-Panthothenate	6.000 ”	Na_2HPO_4	185.00 ”
p-Aminobenzoic acid	20.000 ”	$MgSO_4$ (H_2O)	70.00 ”
Meso-inositol	100.000 ”	$MnSO_4$ (H_2O)	4.50 ”
Folic acid	0.400 ”	Fe-citrate $(H_2O)_5$	4.35 ”
Vitamin B_{12}	0.004 ”	$ZnSO_4$	0.75 ”
Cholinetatrate	434.400 ”	$CuSO_4$	0.37 ”
α-Tocopherole acetate	2.400 ”	KJO_3	0.03 ”
Menadione	1.000 ”	TiO_2	25.00 ”
(Vitamin A 320 000 I.E.)			
(Vitamin D 32 000 I. E.)			
Cellulose	412.000 ”		

Table 3. Essential amino acid composition of the fish meal.[1]

Amino acid	Amino acid content g/kg	Requirement[2] g/kg protein
Arginine	52	60
Phenylalanine+Tyrosine	66	51
Histidine	19	17
Isoleucine	40	25
Leucine	74	39
Lysine	81	50
Methionine+Cystine	41	15
Threonine	42	22
Tryptophan	11	5
Valine	46	32

[1] Analysed on a Technicon amino acid analyser.
[2] Shanks at al. (1962).

Table 4. Analyses of dry matter, protein and fat in the fish. Averages of 10—20 fish per group.

Expt group	PE/TE	Dry matter g/kg±s.d.[1]		Protein in d.m. g/kg±s.d.		Fat in d.m. g/kg±s.d.	
Start	—	248	*6.5*	604	*6.0*	199	*18.3*
1	0.307	297	*11.0*	482	*18.3*	383	*22.4*
2	0.408	280	*5.9*	532	*19.9*	329	*17.2*
3	0.508	264	*3.9*	566	*27.9*	280	*9.8*
4	0.606	250	*4.8*	620	*11.5*	230	*5.3*
Start	—	252	—	658	*12.1*	216	—
5	0.343	302	*6.3*	541	*15.4*	345	*13.3*
6	0.368	298	*6.2*	540	*22.1*	362	*19.9*
7	0.388	299	*3.4*	561	*10.6*	318	*11.8*
8	0.417	289	*5.9*	571	*7.3*	310	*15.1*
Start	—	290	*1.4*	623	*25.5*	281	*12.7*
9	0.343	306	*16.4*	576	*18.7*	291	*15.4*
10	0.368	311	*12.4*	550	*19.7*	322	*22.1*
11	0.388	304	*15.0*	576	*18.0*	291	*13.6*
12	0.417	293	*4.2*	551	*24.4*	301	*22.9*

[1] Standard deviation.

of the fish increased above the start values in all three experiments. There was a nearly linear relation between the fat content of the diet and that in the fish in experiment I. The differences in fat between the groups were highly significant ($P<0.01$). In experiment I an increase in dry matter parallel to the fat increase can be seen from table 4.

Table 5. Weight gain, protein retention, feed efficiency and productive protein value. Averages of duplicate feeding experiments.

Expt. group	Start weight (g)			Final weight (g)			Weight gain and protein retention (g)			Feed intake (g)		Feed effic. [1]	Prod. prot. value[2]	PE/TE
	Whole	Dry matter	Prot.	Whole	Dry matter	Prot.	Whole	Dry matter	Prot. retn.	Dry matter	Prot.			
1	5.57	1.38	*0.83*	8.73	2.59	*1.25*	3.16	1.21	*0.42*	3.45	*1.11*	.351	*.378*	*.307*
2	5.80	1.44	*0.87*	9.99	2.80	*1.49*	4.19	1.36	*0.62*	5.43	*2.16*	.250	*.287*	*.408*
3	5.40	1.34	*0.81*	9.07	2.39	*1.36*	3.67	1.05	*0.55*	4.61	*2.13*	.228	*.258*	*.508*
4	5.88	1.46	*0.88*	8.14	2.03	*1.26*	2.26	0.57	*0.38*	3.42	*1.77*	.167	*.215*	*.606*
5	24.1	6.07	*4.00*	42.7	12.88	*6.97*	18.6	6.81	*2.97*	22.2	*7.81*	.307	*.380*	*.343*
6	24.9	6.27	*4.13*	45.6	13.57	*7.33*	20.7	7.30	*3.20*	22.8	*8.44*	.320	*.379*	*.368*
7	23.9	6.02	*3.96*	43.7	13.05	*7.32*	19.8	7.03	*3.36*	22.9	*8.80*	.307	*.382*	*.388*
8	23.9	6.02	*3.96*	42.2	12.20	*6.96*	18.3	6.18	*3.00*	23.8	*9.62*	.260	*.312*	*.417*
9	68.6	19.89	*12.39*	82.3	25.17	*14.50*	13.7	5.28	*2.11*	31.7	*11.14*	.167	*.189*	*.343*
10	68.1	19.75	*12.30*	95.5	29.68	*16.33*	27.4	9.93	*4.03*	39.4	*14.58*	.252	*.276*	*.368*
11	68.1	19.75	*12.30*	92.3	28.06	*16.16*	24.2	8.31	*3.86*	38.9	*14.98*	.214	*.258*	*.388*
12	69.2	20.07	*12.50*	96.0	28.13	*15.50*	26.8	8.06	*3.00*	34.6	*14.01*	.233	*.214*	*.417*

[1] Weight gain over feed intake (dry matter).
[2] Protein retention over protein intake.

Table 6. Feed consumed per kg fish producted.

Group	PE/TE	Dry feed (kg)	Protein (kg)	Energy (MJ)
1	0.307	1.092	0.351	18.78
2	0.408	1.296	0.516	20.70
3	0.508	1.256	0.580	18.75
4	0.606	1.513	0.783	21.23
5	0.343	1.194	0.420	20.04
6	0.368	1.101	0.408	18.12
7	0.388	1.157	0.444	18.79
8	0.417	1.301	0.526	20.67
9	0.343	2.314	0.813	38.83
10	0.368	1.438	0.532	23.67
11	0.388	1.607	0.619	26.10
12	0.417	1.291	0.523	20.51

Smaller differences were observed in experiments II and III, and maximal fat depositions were seen at a PE/TE-level of 0.368.

Starting and final weights of the fish, together with values from tables 1 and 4 were used to compile table 5. *The protein retention* of the fish is given as the analysed protein content of the fish in each group at the end of the four weeks minus the protein content of the analysed fish samples at the start of the experiments. Two ratios were calculated: *Feed efficiency* is the dry weight gain over the total dry feed intake and the *productive protein value* is the protein retention over the total protein intake.

Table 5 shows maximal protein retentions at PE/TE-values of 0.408 for experiment I, 0.388 for experiment II and 0.368 for experiment III. From this point of view, therefore, the optimal protein content of the diet should lie around 37 to 41% calculated on energy basis. The feed efficiency decreased with increasing protein content of the feed in experiment I, whereas experiments II and III showed maximal values at a PE/TE-value of 0.368. Similarly the productive protein value decreased with increasing protein content of the feed in experiment I, showed constant values at PE/TE-values from 0.343 to 0.388 in experiment II and maximal value at 0.368 in experiment III. These two ratios, therefore again point to PE/TE-values around 0.37 for optimal feeding.

The fish used in experiment III, weighing 68 g at the start, had a comparatively low feed intake and weight gain. Particularly group 9 showed a very low weight increase and weak results. (Table 5.) No explanations can be given for this discrepancy. In experiment I the mortality decreased from 17% in group 1 to zero in group 4. There was no mortality in experiment II, whereas experiment III had a mortality between 5 and 15% during the four weeks.

Lastly, Table 6 gives the feed, protein and energy intakes per kg fish produced. The average values for all groups except 9 were: 1.3 kg dry feed and 20.3 MJ or 4940 kcal per kg fish produced.

DISCUSSION

The energy content of a normal fish diet derives mainly from protein and fat and of these, protein may be the major component giving up to 70% of the total energy consumed. The fish utilizes the protein for maintenance, growth and energy. The requirement for maintenance increases with the size of the fish, and will influence the protein quantity available for growth (Gerking, 1971). The fat content of the diet is utilized as energy and storage. Increasing fat storage will also increase the dry matter content of the fish.

In this work, three criteria were used to measure the growth value of the diets, i.e. the feed efficiency, based on dry weight gain and feed intake, the protein retention as found by analyses of diets and fish, and the productive protein value of the diets. These three values are compared graphically in fig. 1, based on the protein energy content of the diets.

The first experiment gave falling efficiencies of feed as well as protein intake with increasing protein content in the feed. There was, however, a distinct optimal protein retention at a PE/TE level of 0.400. Experiments 2 and 3 represent a further narrowing of the area around 40% protein energy. One must take into consideration that group 9, experiment 3 may be abnormal but even so, these experiments point clearly to an optimum protein level between the PE/TE-values of 0.350 and 0.400.

Combs et al. (1962), Fowler et al. (1964), both working with Chinook salmon (*Onchorynchus tshawytsha*), indicated that the best dietary composition was a 1:2 relationship between protein energy and total dietary energy. Zeitoun (1973) found maximal protein retention for rainbow trout at a dietary level of 0.4 kg protein per kg fed which corresponds to 0.41 PE/TE using the energy factors of this study. Ogino et al. (1976) reported that the protein requirement for maximal growth rate in rainbow trout agreed with the results of Lee and Putnam (1973) on a dietary basis of 36% protein. On energy basis, however, the diets used by Ogino contained higher protein levels. Satia (1974) found a protein requirement of 40% on dietary basis (0.41 PE/TE) using rainbow trout.

Evidently the rainbow trout can use lipid to cover the energy requirement with decreased protein levels. This protein sparing effect has been observed by many authors (Phillips et al., 1964; Atherton and Aitken,

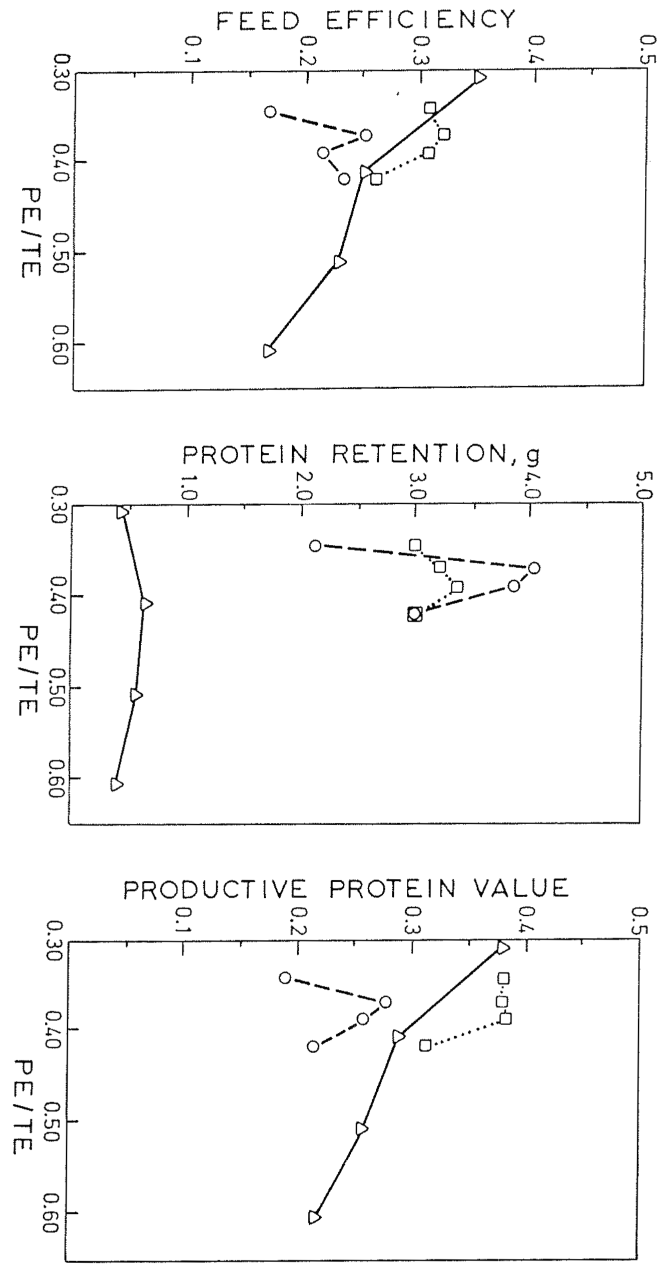

Fig. 1. Feed efficiencies, protein retentions and productive protein values, related to the protein energy content of the feed.
△ Expt. I; □ Expt. II; ○ Expt. III.

1970), but it has often been assumed that larger amounts of fat in the food is harmful to the fish. There are, however, reasons to believe that fat of good quality will bring no harm to the fish (HIGASHI et al., 1964.) ATHERTON (1975) pointed out that the state, level and temperature ta which the fat is fed must be considered. When fish were kept at 12 and 16°C, growth depression was observed at fat levels above 15% in the diet. In our experiments growth was maximal with diets containing about 200 g capelin oil/kg dry diet, at higher fat levels growth depression was indicated. This may possibly also be an effect of restrictions in the amino acid supplement. LEE and PUTNAM (1973) reported highest weight gain and best protein utilization with diets containing 24% fat.

HIGUERA et al. (1977) found an increase in productive protein value from 0.23 to 0.38 by increasing the dietary fat from 6.7% to 18.0%

According to MERTZ (1969) the arginine requriement of salmonoid fishes is high because they lack the urea cyclus. The arginine content in the fish meal used in our experiments was below the requirement of the trout (Table 3.) The availability of the amino acids from the protein source are influenced by the production method and treatment of the meal. The fish meal in these experiments was, as earlier mentioned, hexane-extracted. NOMURA et al. (1972) have reported that factors which had restrictive effects on growth were removed with extraction solvents like hexane. COWEY et al. (1971, 1974) reported that a fish protein concentrate, «Protanimal», obtained by solvent extraction of fish meal, gave poor growth in nutrition experiments with plaice (*Pleuronectes platessa*). BERGSTRØM (1973) found that «Protanimal» gave good growth results on Atlantic salmon.

Commercial Norwegian dry feeds for trout contain about 0.45 to 0.55 PE/TE and this is unnecessarily high according to the results presented. In practical feeding, levels of 0.35 to 0.45 PE/TE are sufficient assuming a balanced protein source and adequate energy supply in the diet.

These experiments were done on small fish (5 to 100 g) which were hand-fed. Energy intakes between 4500 and 5000 kcal were required per kg fish produced, values which are comparable to good practical feeding.

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