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COMPARISONS BETWEEN UNCONVENTIONAL PROTEINS AND FISH MEAL AS A DIETARY NITROGEN SOURCE FOR RAINBOW TROUT (*Salmo gairdneri*): EFFECTS ON *in vitro* MUSCLE PROTEIN SYNTHESIS.

By

GRETHE ROSENLUND*

Institute of Nutrition
Directorate of Fisheries
N-5013 Bergen, Norway

ABSTRACT

Duplicate groups of rainbow trout (*Salmo gairdneri*) were fed different non-animal proteins or fish meals as the sole dietary protein source for a total of 3 weeks considering the two initial weeks to be an acclimatization period. Besides the fish meals NorSeaMink (NSM) and the low temperature dried NorSeaMink-LT (NSM-LT), the dietary proteins included a leaf protein concentrate (LPC), the blue-gree alga *Spirulina* and a yeast product called Toprina. The fish refused to eat diets containing LPC-meal probably due to its strong herbal odour, whereas the other diets were well accepted. The nutritional value of the proteins was assessed by determining the amino acid incorporating activity *in vitro* by ribosomes isolated from skeletal muscle. The ability of Toprina to support cell-free protein synthesis was similar to fish meals whereas the rate of protein synthesis in *Spirulina* fed fish was only 65% of that found in fish given NSM. Thus Toprina seems to be a more efficient fish meal replacer than *Spirulina* in trout diets. In contrast the suitability of LPC appears to be rather limited. The results are also discussed in relation to the amino acid composition of the dietary proteins. The drying temperature used during processing of fish meals was found to influence the availability of dietary amino acids as indicated by the higher rate of muscle protein synthesis in fish fed the low temperature dried meal.

INTRODUCTION

Fish meal is the major protein source in feeds for fish farming. As it is an expensive item in the feed, other protein sources from vegetables, micro-

* Present adress: The Foundation of Scientific and Technical Research, Department of Technical Chemistry, N–7034 Trondheim-NTH, Norway.

organisms and slaughter-house byproducts have been suggested as cheap alternatives (PFEFFER, 1982). However, information concerning the biological value of such products in fish feeds is scarce. Growth studies, most frequently used to evaluate the biological value of proteins, are rather time consuming in fish, thereby limiting the number of products that can be investigated. Comparisons of the amino acid pattern of the test protein with that of fish egg (KETOLA, 1982) or of fish muscle (COWEY and LUQUET, 1983) may indicate the biological value of the meal, but protein digestibility and amino acid availability are not discovered by this method. For instance, SANDHOLM et al., (1976) found that the protein digestibility in rainbow trout was very sensitive to trypsin inhibitors present in raw soybeans. Heat damage during processing may affect dietary amino acid availability (VARNISH and CARPENTER, 1975).

The incorporation of amino acid into protein by ribosomes isolated from skeletal muscle of saithe (*Pollachius virens*) and rainbow trout is significantly correlated to the *in vivo* growth rates (ROSENLUND and LIED, 1984). This *in vitro* method has been used to determine the optimal dietary level of protein energy relative to total energy for maximal growth in different fish species (LIED and ROSENLUND, 1984; ROSENLUND and LIED, 1984). LIED et al. (1983) also demonstrated that the capacity of muscle protein synthesis responded rapidly to changes in feeding rate. In fish starved for 8 days they found that the incorporating activity was nearly back to normal within 12 hrs of refeeding. In rats the effect of processing on the protein quality of dairy products has been determined using this *in vitro* technique (von der DECKEN et al., 1979).

The aim of the present study was to determine the palatibility and nutritional value of some novel proteins relative to fish meal as the sole protein component in trout diets. The three unconventional products used contained relatively high amounts of crude protein (55–65% of dry wt.), and this was necessary to formulate diets satisfying the high protein requirement of fish. The capacity of protein synthesis *in vitro* by muscle polyribosomes of the experimental fish was determined, and the results presented as the amino acid incorporating activity per g wet weight of tissue or per mg ribosomal RNA and discussed in relation to the amino acid composition of the meals.

MATERIALS AND METHODS

FEEDING EXPERIMENTS

Rainbow trout (*Salmo gairdneri*) were supplied by the Aquaculture research Station, Matre[1]), transported to the station in Austevoll[1]) and acclimatized to

[1]) Both situated in Western Norway, and belonging to the Institute of Oceanography, Directorate of Fisheries.

the experimental conditions. Fish with an average weight of 45 g were distributed into 10 circular glassfibre tanks (200 l) supplied with running UV-treated water holding a temperature of 6.5±0.5°C and a salinity of 23±1‰. Each group consisted of 35 fish, and each diet given to two groups.

The dietary protein sources used in the present study were: Two different fish meals, NorSeaMink (NSM)[2]) and the low temperature dried product NorSeaMink-LT (NSM-LT)[2]); the yeast meal Toprina (TOPR) (B.P. Proteins Ltd.), all gifts from Mr. K.E. Gulbrandsen, Norwegian Herring Oil and Meal Industry Research Institute, Bergen, Norway; a meal from the blue-green alga (*Spirulina*) (SPIR) purchased from Sosa Texoco S.A., Mexico; and a leaf protein concentrate (LPC) prepared from lucerne (*Medicago sativa*), a gift from Dr. R. Carlsson, University of Lund, Sweden.

Isonitrogenous (N × 6.25) and isocaloric (14.7 MJ metabolizable energy per kg dry matter) diets were formulated using the test meals as the protein source and cellulose powder as an inert filler substance (Table 1).The energy levels of protein, lipid and digestible carbohydrate were calculated to 35, 55 and 10%, respectively, of the total energy content of the feed. Each diet was given as moist pellets to duplicate batches of fish for 3 weeks. The fish were fed to satiety twice daily. The diets were well accepted except for the one containing LPC-meal. The capacity of protein synthesis was therefore not determined in the LPC-groups.

Table 1. Composition of the experimental diets.

	Protein source				
(g/kg dry wt.)	NSM	TOPR	NSM–LT	SPIR	LPC
Protein meal	403	503	393	454	445
Capelin oil	206	212	212	225	195
Wheat starch[1]	167	167	167	167	167
Cellulose powder	205	72	209	135	174
Vitamin mixture[2]	4	4	4	4	4
Mineral mixture[3]	5	5	5	5	5
Guar gum	10	10	10	10	10

[1] The precooked wheat starch contained 71% digestible carbohydrates.

[2] Composition of the vitamin mixture (g vitamin/kg vitamin mixture): Thiamine-HC1 2.5; riboflavin 5.0; pyridoxine 2.5; Ca-pantothenate 10.0; niacin 37.5; folic acid 1.25; ascorbic acid 100; vitamin A and vitamin D as Rovimix AD. Type 500/100 1.0; α-tocopherol acetate as Rovimix E-50 Adsorbate 15.0.

[3] Commercial standard, mineral mixture used for poultry and swine, composition given previously (LIED and ROSENLUND, 1984).

[2]) From Norsildmel, Bergen.

At the end of the experiment white muscle tissue was dissected from 20 fish in each group and immediately frozen between solid CO_2-blocks as described previously (LUND and von der DECKEN, 1980). Care was taken (by control of stomach content) to obtain muscle samples from fish that were actively feeding.

ANALYTICAL METHODS

Ribosomes and liver cell sap were prepared as described previously (LIED et al., 1982; ROSENLUND et al., 1983; LIED et al., 1983). The amino acid incorporation by ribosomes isolated from 2.5 g muscle tissue pooled from 4 fish was determined as follows. The incubation mixture contained phosphocreatine 25 mM, creatine phosphokinase (EC 2.7.3.2) 0.80 units, ATP 1 mM, GTP 0.1 mM, a mixture of amino acids excluding the radioactive one 0.1 mM, L-(1-^{14}C) phenylalanine (1.85 kBq) 0.006 mM, sucrose 250 mM, Tris-HCl (pH 7.6 at 25°C) 35 mM, KCl 80 mM, $MgSO_4$ 4 mM, liver cell sap equivalent to 0.5 mg protein, and 50 microl of the ribosome suspension corresponding to 25–30 microg rRNA. The final incubation volume was 130 microl. After incubation for 4 min at 28°C, 100 microl of the incubation mixture were transferred to filter-paper discs, which were kept for 10 min in ice-cold 10% trichloroacetic acid, washed 3 times in ethanol, and finally dried and counted for radioactivity in a scintillation spectrometer.

Four determinations were made with each ribosome suspension. The results were evaluated using one way analysis of variance (SOKAL and ROHLF, 1969). The capacity of protein synthesis was expressed as activity per g wet weight of muscle tissue and per mg of ribosomal RNA. All results are given as the mean ±standard of error of the mean (S.E.M.).

RNA contents were determined by extracting the ribosomes in 0.4 M $HClO_4$ for 18 min at 70°C, and the absorbance measured at 260 nm. RNA was calculated on the basis of 34.2 optical density units at 260 nm/mg of RNA. After hydrolysis of proteins in 6 N HCl for 20 hrs at 110°C amino acids were analysed on an Amino Analyzer Liquimat III (Kontron) using norleucine as an internal standard. Tryptophan was determined separately by a fluorimetric method after hydrolysis with $Ba(OH)_2$ (BUTTERY and SOAR, 1975; SACHSE, 1981).

RESULTS AND DISCUSSION

During an acclimatization period of 2 weeks the fish adapted well to all the experimental diets except that containing LPC-meal. Omitting the LPC-groups that refused eating, daily feed intake during the third week of the experimental period was similar in the remaining eight groups amounting to 8

gram dry feed per kg wet fish. Loss of appetite in fish may be ascribed to dietary amino acid deficiencies (KETOLA, 1982) but the feed response is probably also influenced by dietary flavour and odour. As the amino acid composition of the LPC-meal was comparable to some of the other protein sources used (Table 3) the refusal of this diet by the rainbow trout apparently was caused by the strong herbal odour of the product. This is consistent with the reduced growth performance and protein utilization found by COWEY et al. (1971) in plaice (*Pleuronectes platessa*) fed diets containing more than 40% of the total protein as leaf protein.

Data on the incorporation of phenylalanine into protein per g wet wt. of muscle tissue and per mg of ribosomal RNA are shown in Table 2. As the protein synthesis assay ranked the test proteins identically in the replicate groups all values obtained for each diet were combined.

The concentration of rRNA was fairly constant in all groups although the rRNA level seemed to be slightly higher in fish receiving the NSM-LT meal than in the other groups. This dietary protein source also resulted in the highest rate of cell-free protein synthesis, 23.9±1.4 pmol PHE incorporated per min and unit of tissue. This value was, however, not statistically different from that obtained in either Toprina or NSM fed fish, whereas diets containing *Spirulina* resulted in a significantly lower capacity of muscle protein synthesis in rainbow trout. Thus the present findings indicate that the nutritional value of Toprina is comparable to that of fish meals whereas the ability of *Spirulina* to support growth in rainbow trout is inferior to that of the other test proteins.

The rate of protein synthesis per g wet wt. of tissue is a function of ribosomal activity and content. The supply of dietary amino acids is known to influence ribosomal functions associated with polypeptides initiation and elongation (MUNRO, 1976), thus playing an important role regulating ribosomal activity. In cod (*Gadus morhua*) starved for three days the specific

Table 2. Effect of protein source on the content of ribosomal RNA and on the incorporation of phenylalanine into protein in vitro in skeletal muscle of rainbow trout[1].

Protein source	mg rRna	pmol PHE incorporated into protein per min per	
	g wet wt.	g wet wt.	mg rRNA
NSM	0.75±0.06	22.0±1.7	30.1±2.0
TOPR	0.77±0.03	22.3±1.8	28.7±2.0
NSM–LT	0.86±0.05	23.9±1.4	28.4±2.4
SPIR	0.76±0.02	14.4±1.3[2]	19.0±1.7[2]

[1] The results are presented as the mean values ± S.E.M. of 10 determinations.
[2] Results are significantly different ($p < 0.05$) from each of the three values above.

Table 3. Comparison of the amino acid composition of the test meals and the amino acid pattern of rainbow trout skeletal muscle (values are expressed as g amino acid per 16 g N).

Amino acid	NSM	TOPR	NSM–LT	SPIR[1]	LPC	Trout muscle
Methionine	2.8	1.8	3.0	1.8	1.9	3.0
Isoleucine	3.2	4.1	3.9	5.1	3.8	4.1
Leucine	7.0	7.4	7.5	7.5	8.9	7.8
Valine	4.3	5.4	4.8	5.7	5.5	5.1
Phenylalanine	3.5	4.0	3.5	3.6	5.3	3.9
Lysine	8.6	8.5	9.7	5.0	7.3	10.8
Histidine	2.1	1.9	2.4	1.3	2.5	2.6
Arginine	6.7	5.8	6.6	7.7	7.0	6.5
Threonine	4.4	5.8	4.9	4.1	5.5	5.2
Tryptophan	1.0	1.4	1.2	1.3	–	–
Aspartic acid	9.7	11.6	10.2	10.6	10.5	10.9
Serine	4.5	5.9	5.1	4.3	5.3	4.5
Glutamic acid	13.8	15.2	14.3	15.5	11.6	15.1
Glycine	6.8	5.2	5.8	4.8	5.7	5.2
Alanine	6.4	7.3	6.4	5.9	6.0	6.4
Tyrosine	3.2	3.4	3.1	3.8	4.3	3.1
Cysteine	1.7	1.8	1.6	0.8	1.6	1.6

[1] The amino acid composition was given by the producer.

ribosomal activity dropped to about 50 % of that in normal fed fish, whereas the ribosome concentration remained unchanged during the same period of time (LIED et al., 1983). The reduced incorporating activity found in *Spirulina* fed fish was mainly due to a decreased ribosomal activity indicating dietary amino acid deficiencies.

The amino acid composition of the test proteins and of rainbow trout skeletal muscle is shown in Table 3. As muscle is the major product formed in young growing fish it has been suggested that the amino acid pattern of muscle could serve as a useful guide to the nutritional requirements (COWEY and LUQUET, 1983). The amino acid content of the two fish meals was quite similar to each other and to the composition in fish muscle. However, the tendency of a higher capacity of protein synthesis as well as an increased concentration of rRNA in muscle obtained with NSM-LT (Table 2) indicate a better bio-availability of the amino acids in this meal. Heat treatment during processing may lead to chemical changes in the proteins, the formation of inter- or intramolecular linkages impeding the function of the proteolytic enzymes, thus reducing protein digestibility (VARNISH and CARPENTER, 1975). Such changes may be difficult to prove be chemical means. The differences found here between the fish meals may therefore primarily be an effect of the different drying temperatures during processing. GULBRANDSEN found that the

nitrogen digestibility in mink was significantly improved from 86 to 89–92% by reducing the drying temperature from 90–95°C for NSM to 70°C for NSM-LT (pers. comm.).

Compared with fish muscle protein the contents of the sulphur containing amino acids were low in all the non-animal proteins. Except for *Spirulina*, however, the contents were well above the requirement of 25–30 g sulphur amino acids per kg dietary protein as determined for rainbow trout by RUMSEY et al. (1983). The low concentration of methionine in Toprina did not seem to suppress fish growth as assessed by the determination of muscle protein synthesis *in vitro*, supporting the findings of RUMSEY et al. (1983).

The *Spirulina* protein also had low levels of lysine and histidine. According to KETOLA (1982) lysine is an important dietary amino acid promoting growth. *Spirulina* contained only 50 g lysine per kg crude protein and the reduced rate of protein synthesis obtained with this protein source may possibly be attributed to a limited supply of lysine, thus agreeing with a requirement of 61 g lysine per kg dietary protein for maximal growth as reported be KETOLA (1982). Recently several reports concerning the lysine requirements of rainbow trout have been published (see WALTON et al., 1984). Although the agreement between estimates is poor, values range from 37 to 50 g lysine per kg protein indicating that *Spirulina* contains adequate amounts of this amino acid. A combination of low levels of several essential amino acids and a restriction of protein digestibility by the resistent cell wall of the alga, may be responsible for the reduced ability of muscle protein synthesis observed in *Spirulina* fed fish.

Taken together, the experiments showed that of the novel proteins tested only Toprina had an acceptability and nutritional value comparable to fish meal. A high level of Torpina may therefore be included in trout rations. Although *Spirulina* was well accepted the lower protein quality of this meal indicated that it should not be added as the major protein component in fish feeds. The LPC-diet was not accepted by the fish and the suitability of this protein concentrate as a fish meal replacer therefore was limited.

In spite of the low acclimatization temperature in this investigation, differences in dietary protein quality affecting growth could be detected by assessment of muscle protein synthesis *in vitro*, after a feeding period of only three weeks. The present results support those obtained by ATACK and MATTY (1979) for rainbow trout fed diets containing Toprina, *Spirulina* or fish meal for 8 weeks. They found that growth and protein utilization expressed as PER were comparable in fish fed either fish meal or Toprina whereas *Spirulina* had a lower digestibility and resulted in a lower growth rate as well as PER. It may therefore be concluded that the determination of amino acid incorporating activity by isolated muscle ribosomes is a rapid and reliable screening-method evaluating proteins feasible for inclusion in commercial fish diets.

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