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STUDIES ON THE NUTRIENT REQUIREMENTS OF RAINBOW TROUT (*SALMO GAIRDNERI*) GROWN IN SEAWATER AND FRESHWATER

by

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SUMMARY

The results of this study indicate that significant differences exist in protein and lipid utilization of Rainbow trout grown in seawater as compared with those reared in freshwater. Approximately 50 % protein was required in the diet for maximum growth, feed efficiency and survival in seawater. A lack of ω3 type fatty acid in the diet caused high mortality of seawater fish. The level of essential amino acids in plasma were not greatly affected by the salinity. However, the levels of taurine, β-alanine, aspartic acid, glutamic acid and glycine were significantly lower in salt water fish as compared with freshwater fish. It appears that fish can derive sodium, potassium and magnesium from the seawater, however, the calcium and phosphorous deposition in the body is reduced by the presence of these minerals in the diet.

INTRODUCTION

In recent years a considerable amount of research has been conducted on freshwater fish nutrition, however, studies on the nutrient requirements of salmonids grown in saltwater are limited. Adaptation of salmonids to hyperosmotic media, consisting of natural seawater or dilution thereof, involves changes in the energy cost of osmoregulation (Rao, 1968) and the absorption of minerals from the body surface and the intestine (Parry, 1966). The dietary requirement of minerals as well as other nutrients may be influenced by the absorption of inorganic ions from a saline environment. Zeitoun *et al.*, (1973) reported that the protein requirement of Rainbow trout (*Salmo gairdneri*) increased with the increase in the water salinity (10 and 20 ppt).

The present work was primarily designed to determine the basic differences in protein and lipid utilization of rainbow trout grown in seawater and freshwater, under similar experimental conditions and the same test diets. Changes in the plasma amino acid level and mineral balance were also studied.

MATERIALS AND METHODS

The composition of the experimental diets is shown in Table 1. Diets 1-3 contained herring oil and the test protein at levels ranging from 40 to 50% of the dry diet. Diets 4-5 contained 40 and 50% test protein and corn oil, the dry portion of the diet was mixed with hot (60°C) water. The gel that formed upon cooling was passed through a meat grinder to obtain the desired size of pellet. The wet diet (60% water) was prepared every 2 weeks and was stored at -20°C until fed.

Approximately 4.5 to 5 inch rainbow trout were distributed in 20 lots of 50 fish each. The weight of each lot was adjusted so that each lot contained an equal weight of fish. The fish were held in 3-foot diameter circular aquaria. Fish in ten aquaria were gradually acclimated to seawater over three weeks. The water temperature was maintained between 15-16°C throughout the experiment in both salt water and freshwater aquaria. The salinity of seawater was 32 ppt. Water temperature was monitored daily and the pH, dissolved oxygen and total ammonia nitrogen measured periodically. The record of mortality was also maintained and dead fish were examined for gross abnormalities.

After the saltwater adaptation and a two week adjustment period, duplicate lots of fish were fed each of the experimental diets for 12 weeks. Fish were fed all they would eat 3 to 4 times a day. They were group weighed every four weeks. During the last week of experiments, fish were fed the test diets containing 0.5% chromic oxide as an inert digestion indicator. Before the fecal collection, fish were lightly anesthetized by immersion in seawater containing MS-222 (Ethyl m-aminobenzoate methanesulfonate). Feces were collected from the rectal region by applying gentle pressure to the surface of fish. Feces samples were freeze dried, ground and analyzed for nitrogen and chromic oxide.

At the beginning and the termination of the experiment, samples were taken for the determination of body composition. Carcass analyses were conducted in triplicate on the pooled sample of 10 fish for each tank after the fish had been frozen, ground, freeze dried and then reground. After the final weight measurement, blood samples were taken for plasma amino acid analysis from fish fed diets containing herring oil (Diets 1 to 3).

Nitrogen in feed, feces and carcass was determined on an automated Kjeldahl analyzer (Technicon Instrument Co. Ltd.) according to the method of Ferrari (1960). The method of Bligh and Dyer (1959) was used for body lipid determination. Chromic oxide in feed and feces was analyzed according to the method of Arthur (1970). Body moisture and ash was estimated by A.O.A.C. (1970) procedure. Ash samples were analyzed for calcium, phosphorous, magnesium, sodium and potassium. Calcium and magnesium were determined using an SP90 Atomic Absorption Spectrophotometer (Unicam Instruments Ltd.) according to the A.O.A.C. (1970) method. Sodium and potassium were measured on the Atomic Absorption Spectrophotometer operating on emmission. Phosphorus was determined by the method of Fiske-Subbarow (1925) and plasma amino acids were analyzed on an automatic amino acid analyzer (Beckman 120C).

The data obtained from this experiment was analyzed by the method of analysis of variance and tests of significance were made using Duncan's multiple range test at the 5% probability level as outlined by Steel and Torrie (1960).

RESULTS AND DISCUSSION

Growth and Feed Efficiency

The data presented in Table 2 clearly indicated that the growth of fish was significantly better in seawater than freshwater. Incorporation of 50% protein in the diet resulted in a significant improvement of weight gain of fish fed either corn oil or herring oil. The diet containing 45% protein produced an increase in weight gain over the 40% protein diet in seawater but such a response was not evident in freshwater. Salt water fish fed the diet containing 50% protein and fish oil showed the maximum weight gain compared with any other group. The performance of fish fed the diet containing 40% protein was extremely poor both in saltwater and freshwater.

Overall feed consumption results show that saltwater fish had significantly higher feed intakes than freshwater fish. The diet containing 50% protein showed better feed efficiency compared with the 40 and 45% protein diets, both in freshwater and saltwater. It appears that a minimum of 50% protein in the diet is essential for maximum weight gain and feed efficiency of rainbow trout reared in seawater on these test diets.

Mortality

Although there was no mortality of freshwater fish during the experiment, fish grown in seawater showed high mortalities (Table 2). Autopsy of these fish revealed extensive inflammation of the digestive tract particularly the intestine. Histological examination showed hyperaemia and sloughing of intestinal epithelia.

Significantly more fish died on the corn oil diet than on the herring oil diet. The high mortalities on corn oil diet were probably attributable to the lack of essential fatty acids ($\omega3$ type fatty acids) in the diet. Evidence indicated that fish cannot synthesize fatty acids of $\omega3$ series, therefore those fatty acids must be provided in the diet. The presence of $\omega3$ type fatty acids in the diet seems to be more critical for saltwater fish than for freshwater fish.

Results also indicate that mortalities were significantly lower in the group fed 50% protein than those receiving 40 and 45% protein. This is in agreement with the findings of Zeitoun *et al.* (1973) that a higher level of protein is required in the diet to protect the fish against the hypertonic external environment.

Digestibility of Protein

The apparent digestibility of dietary protein decreased significantly with the increase in salinity for fish fed herring oil. This decrease was consistent with all the three levels of protein. However, such decreases in protein digestibility were not observed for fish receiving the corn oil diet.

There was an improvement in the digestibility of protein with the increase in the level of protein. This increase in the digestibility of protein was probably due to the lower level of carbohydrate (dextrin) in the high protein diets (Table 1). Kitamikado *et al.* (1964) observed that incorporation of a high level of starch in test diets reduced the digestibility of protein.

Body Composition

The effects of dietary treatment on body composition of rainbow trout are shown in Table 4. Generally, the moisture, lipid and ash content of fish were not significantly influenced by the salinity. However, a higher carcass protein level was noted in fish grown in saltwater than those reared in freshwater.

Both freshwater and saltwater fish fed 50% protein showed higher carcass lipid and lower moisture levels than fish receiving 40 and 45% protein. On the other hand, the carcass protein was not influenced by the increase in dietary protein level. Ogino and Saito (1970) also observed that in carp (*Cyprinus carpio*) the accumulation of protein in the body reached a maximum value when the dietary protein level was about 38% but the body weight increased up to the highest level of protein used (55%). It appears that rainbow trout convert additional protein to body fat.

Although feeding corn oil and herring oil to fish did result in a consistent change in body protein, moisture and ash level, the body lipid content was always greater in the corn oil fed group than in the group receiving herring oil. This response was consistent for fish kept in either freshwater or saltwater.

Plasma Amino Acid Level

Plasma amino acid data (Table 5) indicates that a higher concentration of taurine was present in freshwater fish than in marine fish fed similar test diets. The biochemical significance of this compound is unknown. Chance *et al.* (1962) reported that the level of taurine was very high in the plasma of spawning male Chinook salmon (*Oncorhynchus tshawytscha*) in freshwater but low in young salmon migrating to the ocean. Cowey *et al.* (1962) reported that the taurine level was high in the plasma of migrating Atlantic salmon (*Salmo salar*) in both freshwater and saltwater. Parry (1961) failed to demonstrate an osmoregulatory function for taurine.

Glycine, glutamic acid, aspartic acid and β-alanine levels were also lower in saltwater fish than in freshwater fish. The concentration of essential amino acids except leucine and isoleucine were not affected by the salinity. Both isoleucine and leucine were low in freshwater fish. In general, no consistent plasma amino acid pattern was noted either in freshwater or saltwater fish with the increase in level of dietary protein.

A large turnover of salt and other minerals takes place in marine fish through the body surface and gut. Evidence indicates that a physiological interrelationship exists between minerals and the nitrogen components of the diet. Savage (1972) indicated that cellular concentrations of alkali minerals especially potassium and sodium have specific effects on protein and amino acid metabolism in chicks. It is possible that the high turnover of the minerals in marine environment may affect the amino acid metabolism and increase the requirement of certain amino acids in fish. This would also explain the high protein intake of saltwater fish. To date, no systematic study has been done on the intermediary metabolism of amino acids in fish.

Mineral Composition

The mineral composition of the fish carcasses is shown in Table 6. Results indicate that calcium and phosphorus levels were lower in saltwater fish than freshwater fish, whereas magnesium, sodium and potassium levels were higher in fish grown in saltwater. The level of dietary protein and type of lipid had no significant effect on the mineral composition of fish.

It seems probable that fish can derive a number of minerals from the seawater to satisfy the specific needs for growth and metabolism. Some workers (Templeton and Braun, 1963; Ichikawe and Oguri, 1961) have reported that calcium and phosphorus are absorbed and excreted through the gill membrane of fish, but phosphate is more efficiently absorbed from the intestinal tract (Philips *et al.* 1959). Recently, Yone *et al.* (1974) have demonstrated that deletion of calcium from the diet did not affect the growth of Seabream (*Chrysophyrs major*). It is possible that the calcium present in the diet may affect the absorption and excretion of both calcium and phosphorous and consequently the retention in the body. At present, experiments are in progress in our laboratory to investigate mineral utilization by salmonids held in seawater.

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TABLE 1. Percentage composition of the experimental diets

	Diet Number				
	1	2	3	4	5
	Dietary Protein Level				
Ingredient	40	45	50	40	50
Casein[1]	30.4	34.2	38.0	30.4	38.0
Gelatin	9.6	10.8	12.0	9.6	12.0
Dextrin	38.0	33.0	28.0	38.0	28.0
Vitamin mixture[2]	2.0	2.0	2.0	2.0	2.0
Mineral mixture[3]	4.0	4.0	4.0	4.0	4.0
Cellulose[4]	6.0	6.0	6.0	6.0	6.0
Herring oil	10.0	10.0	10.0	0	0
Corn oil	0	0	0	10.0	10.0

[1]Vitamin-free casein. Product of Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A.

[2]Vitamins added to supply the following (mg or I.U./kg of diet): Thiamine HCl, 50; riboflavin, 200; pyridoxine HCl, 50; d-calcium pantothenate, 300; d-biotin, 5; folic acid,15; niacin, 500; cyanocobalamin, 0.2; L-ascorbic acid, 1000; inositol, 2000; choline chloride, 5000; para amino benzoic acid, 400; vitamin A, 5000 I.U.; vitamin D_3, 1500 I.U.; DL-alpha-tocopherol acetate, 300 I.U.; vitamin K, 40; butylated hydroxy toluene, 22.

[3]Modified Bernhart-Tomarelli salt mixture. Composition is described in Nutrient requirements of domestic animals, No. 11, Nutritional Requirements of Trout, Salmon and Catfish, National Academy of Sciences, Washington, D.C. 1973, p.49.

[4]Carboxy methyl cellusose (2%) and α-cellulose (4%).

TABLE 2. Average body weight, weight gain, feed conversion and mortality of fish fed various experimental diets.

	Dietary Protein Level				
	40 (H.O.[1])	45 (H.O.)	50 (H.O.)	40 (C.O.[2])	50 (C.O.)
			<u>Fresh Water</u>		
Average initial weight, g	50.7	50.5	51.1	50.6	50.9
Average final weight, g	92.6	94.3	103.7	93.7	99.1
Average weight gain, %	82.6	86.7	102.9	85.2	94.7
Feed : Gain Ratio	1.25	1.21	1.03	1.26	1.05
Mortality, %	0	0	0	0	0
			<u>Sea Water</u>		
Average initial weight, g	51.1	50.9	51.2	51.5	50.9
Average final weight, g	94.9	104.5	118.8	95.9	112.9
Average weight gain, %	85.7	105.3	132.0	86.2	121.8
Feed : Gain Ratio	1.36	1.30	1.14	1.38	1.16
Mortality, %	15.6	16.7	9.7	25.0	15.5

[1]H.O. = Herring oil, [2]C.O. = Corn oil

TABLE 3. Digestibility of protein in fresh water and sea water

Treatment	Fresh water	Sea water
	(%)	(%)
Herring oil		
+ 40% protein	90.4 ± 0.76[1]	88.4 ± 0.82
+ 45% protein	93.3 ± 0.88	89.5 ± 0.54
+ 50% protein	94.7 ± 0.76	92.1 ± 0.91
Corn oil		
+ 40% protein	89.7 ± 1.14	89.5 ± 1.34
+ 50% protein	91.7 ± 0.90	92.9 ± 0.58

[1]Mean ± standard deviation.

TABLE 4. The body composition of fish fed various experimental diets.

Treatment	Percentage			
	Moisture	Protein	Lipid	Ash
Initial	75.3 ± 0.21[1]	14.9 ± 0.33	7.5 ± 0.27	2.35 ± 0.12
		Fresh water		
Herring oil				
+ 40% protein	72.3 ± 0.06	15.3 ± 0.26	9.9 ± 0.29	2.54 ± 0.05
+ 45% protein	72.3 ± 0.48	15.4 ± 0.19	9.7 ± 0.49	2.58 ± 0.08
+ 50% protein	70.7 ± 0.28	15.2 ± 0.11	11.6 ± 0.20	2.44 ± 0.02
Corn oil				
+ 40% protein	72.3 ± 0.09	15.1 ± 0.21	11.1 ± 0.18	2.50 ± 0.05
+ 50% protein	70.8 ± 0.44	15.2 ± 0.13	11.6 ± 0.42	2.44 ± 0.02
		Sea water		
Herring oil				
+ 40% protein	71.6 ± 0.05	16.0 ± 0.27	9.8 ± 0.28	2.60 ± 0.06
+ 45% protein	71.3 ± 0.09	15.9 ± 0.23	10.2 ± 0.24	2.58 ± 0.07
+ 50% protein	70.9 ± 0.34	16.6 ± 0.21	11.0 ± 0.38	2.54 ± 0.06
Corn oil				
+ 40% protein	71.5 ± 0.19	16.1 ± 0.22	9.9 ± 0.31	2.48 ± 0.06
+ 50% protein	70.1 ± 0.14	15.7 ± 0.18	11.8 ± 0.32	2.42 ± 0.08

[1] Mean ± standard error.

TABLE 5. Effect of dietary protein level on plasma amino acid content of freshwater and sea water rainbow trout.

Amino acid	Fresh Water Protein Level			Sea Water Protein Level		
	40	45	50	40	45	50
	(μ moles/ml of plasma)					
Taurine	3.723	3.690	3.819	1.428	1.660	1.542
Aspartic acid	0.238	0.259	0.198	1.177	0.152	0.109
Threonine	0.441	0.465	0.465	0.540	0.493	0.512
Serine	0.550	0.622	0.584	0.515	0.385	0.385
Asparagine and/or glutamine	0.455	0.468	0.507	0.529	0.424	0.411
Proline	1.290	1.512	1.400	1.229	0.948	1.062
Glutamic acid	0.751	0.894	0.842	0.536	0.521	0.569
Glycine	3.190	2.979	3.063	1.779	1.601	1.645
Alanine	1.096	0.997	1.092	1.204	1.184	0.919
α-amino-n-butyric acid	0.032	0.040	0.052	0.042	0.031	0.032
Valine	1.062	1.045	1.035	1.000	0.984	1.059
Methionine	0.165	0.214	0.185	0.219	0.182	0.263
Isoleucine	0.337	0.389	0.376	0.415	0.434	0.509
Leucine	0.625	0.733	0.734	0.820	0.812	0.902
Tyrosine	0.179	0.209	0.207	0.255	0.212	0.216
Phenylaline	0.199	0.235	0.245	0.287	0.226	0.240
β-Alanine	0.195	0.185	0.207	0.107	0.081	0.039
Lysine	0.586	0.633	0.657	0.664	0.564	0.617
Histidine	0.156	0.172	0.159	0.182	0.169	0.185
Arginine	0.250	0.256	0.197	0.234	0.208	0.203

TABLE 6. Mineral composition of Rainbow trout

Treatment	Calcium	Phosphorous	Magnesium	Sodium	Potassium
		(mg/g of carcass)			
		Fresh water			
Herring oil					
+ 40% protein	5.59 ± 0.04[1]	4.66 ± 0.08	0.27 ± 0.01	0.76 ± 0.04	2.09 ± 0.13
+ 45% protein	5.53 ± 0.10	4.70 ± 0.14	0.29 ± 0.01	0.75 ± 0.04	2.19 ± 0.06
+ 50% protein	5.27 ± 0.12	4.66 ± 0.06	0.26 ± 0.01	0.74 ± 0.02	2.17 ± 0.06
Corn oil					
+ 40% protein	5.51 ± 0.15	4.68 ± 0.14	0.27 ± 0.00	0.72 ± 0.02	2.15 ± 0.11
+ 50% protein	5.43 ± 0.21	4.69 ± 0.21	0.28 ± 0.01	0.74 ± 0.03	2.15 ± 0.07
		Sea Water			
Herring oil					
+ 40% protein	5.00 ± 0.26	4.60 ± 0.09	0.38 ± 0.01	0.87 ± 0.04	2.62 ± 0.08
+ 45% protein	4.86 ± 0.22	4.36 ± 0.15	0.36 ± 0.00	0.84 ± 0.04	2.54 ± 0.10
+ 50% protein	4.84 ± 0.09	4.32 ± 0.08	0.36 ± 0.01	0.85 ± 0.08	2.63 ± 0.14
Corn oil					
+ 40% protein	4.83 ± 0.21	4.34 ± 0.07	0.34 ± 0.01	0.86 ± 0.04	2.45 ± 0.16
+ 50% protein	4.65 ± 0.13	4.15 ± 0.06	0.34 ± 0.01	0.84 ± 0.03	2.43 ± 0.07

[1]Mean ± standard error.