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## NUTRITIVE VALUE FOR GROWTH OF SOME FISH PROTEINS

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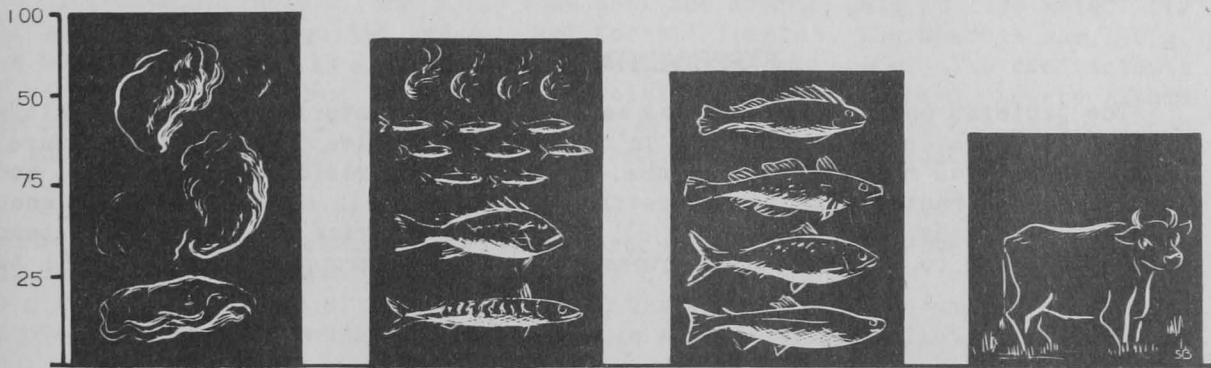
### ABSTRACT

Feeding studies were conducted with growing rats to determine the approximate nutritive value of proteins extracted with acetone from the edible flesh of 17 species of fish. No statistically significant differences were found in the gain in weight of groups of rats fed the various proteins. It is concluded that all proteins tested are of good nutritive quality, especially so far as balancing the average diet is concerned. The proteins tested were also found to be well digested.

### INTRODUCTION

Comparatively few studies have been conducted to determine the nutritive value of proteins derived from fishery sources. Lanham and Lemon (1938) reviewed the literature and found that, in general, earlier investigators had rated proteins from fishery sources about equal to that of beef. Lanham and Lemon also reported experimental work on feeding studies with growing rats, using protein obtained by extraction with acetone of the edible flesh of certain fishery products. The proteins could be classified into the following groups based on comparative gain in live weight estimated from equal protein intake. If the protein of oysters is arbitrarily rated 100; the proteins of pilchard, red snapper, shrimp, and Boston mackerel rated about 90; those of shad, cod, croaker, and coho salmon rated about 80; and that of beef rated 63. These comparative values indicate that the proteins of the several fishery products are of somewhat higher quality than beef.

Another index for determining the comparative nutritive value of proteins depends on assaying the metabolic use of the proteins, instead of measuring the



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growth of animals fed a limited quantity of protein. This index of nutritive value is designated "biological value" of a protein. It is determined by feeding adult male rats (biological value for maintenance) on a protein free diet and determining excretion of protein in urine and feces. The test protein is then fed in limited quantity and the excretion is determined. The index of biological value is calculated from the formula: food nitrogen intake minus (fecal nitrogen when protein was fed minus fecal nitrogen when protein free diet was fed) minus (urinary nitrogen when protein was fed minus urinary nitrogen when protein free diet was fed) divided by food nitrogen intake minus (urinary nitrogen when protein was fed minus urinary nitrogen when protein free diet was fed). The answer is multiplied by 100 to give comparative values having 100 as the value of the highest quality protein.

Lanham, Lee, and Nilson (1940) reported that the biological value of the protein from similarly prepared flesh of Dungeness, king, and blue crabs, ranged from 68.9 to 78.3, while that of beef round equaled 70.2. They concluded that the data showed the biological value of the protein of crab meat equals or is slightly superior to that of beef round. The corrected digestibility values of the proteins were found to range from 85.6 to 93.3 percent on a scale in which 100 percent equals perfect digestion. These data indicate very satisfactory digestibility. Unpublished preliminary data from this laboratory show that the biological value of the protein prepared by acetone extraction of oysters is about 95.4. These data apparently confirm the feeding studies with rats as to the very good nutritive value of oysters.

In some respects, it would have been very desirable to gather more data on the biological value of proteins from other fishery products, but the many chemical analyses required did not permit doing this type of assay with the staff available. It was decided, therefore, to conduct further feeding tests with growing rats to determine the comparative nutritive value of the proteins previously prepared for amino acid studies reported by Pottinger and Baldwin (1939). These proteins were also of the same series as those used in the rat feeding experiments reported by Lanham and Lemon (1938).

There was a second consideration which dictated choice of experimental method; namely, less concern was felt for determining the "true" nutritive values of these proteins, than to get an estimate of probable "balancing" values in the diet. The diet fed to the rats contained 9.0 percent protein derived from fish flesh, and 1.2 percent from supplementary sources such as liver extract, dried yeast, wheat germ, and corn starch. The supplementary proteins amounted to about 12 percent of the total protein content.

These tests were begun after the war started in order to have available on request, data on the comparative nutritive value of fish proteins. The data have been reported to interested government agencies, but have not been previously published.

#### EXPERIMENTAL METHODS

The proteins used in these studies were prepared by covering the ground edible flesh with acetone, and permitting it to remain in this solvent for about 10 hours. This procedure was repeated three times. The resulting residue was reground, and continuously extracted with fresh acetone for 14 hours in an extractor designed by Lemon, Griffiths, and Stansby (1936). The solid material was dried on a steam bath in open air to vaporize the acetone. It was then ground in a Wiley mill to

light, dry meal which could be stored indefinitely in sealed Mason jars. The crude protein content varied from 86 to 97 percent, as reported in Table 1.

Table 1 - Source and Crude Protein Content of the Acetone Extracted Fish Flesh

| Source of Protein                            | Crude Protein (N x 6.25) | Source of Protein                            | Crude Protein (N x 6.25) |
|--|--------------------------|--|--------------------------|
|  | Percent                  |  | Percent                  |
| Beef round .....                             | 91.28                    | Salmon (Continued):                          |                          |
| Bonito ( <i>Sarda chiliensis</i> ) .....     | 93.19                    | Coho ( <i>O. kisutch</i> ) .....             | 94.44                    |
| Catfish ( <i>Ictalurus punctatus</i> ) ..    | 85.50                    | Pink ( <i>O. gorbuscha</i> ) .....           | 95.28                    |
| Halibut ( <i>Hippoglossus hippoglossus</i> ) | 88.94                    | Sockeye ( <i>O. nerka</i> ) .....            | 93.13                    |
| Herring:                                     |                          | Squeteague ( <i>Cynoscion regalis</i> )      | 87.81                    |
| Lake ( <i>Leucichthys arctedi</i> ) ....     | 87.68                    | Trout, lake ( <i>Cristivomer namaycush</i> ) | 93.31                    |
| Sea ( <i>Clupea harengus</i> ) .....         | 86.01                    | Tuna:  |                          |
| Mullet ( <i>Mugil species</i> ) .....        | 90.87                    | Albacore ( <i>Germo alalunga</i> ) ...       | 95.75                    |
| Salmon:                                      |                          | Bluefin ( <i>Thynnus thynnus</i> ) ...       | 94.50                    |
| Chinook ( <i>Oncorhynchus tshawytscha</i> )  | 93.38                    | Skipjack ( <i>Katsuwonus pelamis</i> )       | 96.50                    |
| Chum ( <i>O. keta</i> ) .....                | 95.35                    | Yellowfin ( <i>Neothunnus macropterus</i> )  | 96.63                    |

Lanham and Lemon (1938) showed that the protein of acetone extracted haddock flesh had approximately the same nutritive value as unextracted flesh that was dried on a steam bath, and the apparent digestibility was similar. The rats fed the treated protein grew about as well as rats from the same colony in other experiments, which were fed a similar basal diet *ad libitum*, but were fed cooked fish in a separate feed cup daily in such quantity that the total protein intakes over the experimental period were equal. There is no reason for believing that the nutritive value of the extracted proteins used was significantly altered either by extraction or prolonged storage.

The diets consisted of enough acetone extracted fish flesh to provide 9 parts crude protein; lard, 8; cod liver oil, 2; salt mixture U.S.P. XI, No. 2, 4; dried brewer's yeast, 2; wheat embryo, 1; liver extract (Lilly), 0.5; and corn starch to make 100 parts by weight. These diets were made up at not more than bi-weekly intervals, and stored in a refrigerator.

The rats were allotted at an initial live weight of 49 to 57 g., into groups of from 10 to 22 animals, using both sexes. The animals were not all allotted at the same time, but in each series, a number of rats adequate to furnish control data were fed the diet containing beef protein. All rats were individually housed in wire screen cages fitted on wire mesh floors. The temperature of the room was maintained at 80° F. They were supplied with food and water *ad libitum*, and weekly records were taken on live weight and food consumption.

### EXPERIMENTAL DATA

The data in Table 2 (see p. 4) show that the average gain in live weight for all groups of males equaled 163 g., and for all females, the average was 130 g. The mean gain for the 18 groups, including both sexes, was 144 g. The coefficients of variability for sub-group and group weights (Table 3, see p. 4) are within expected limits for this type of experiment, and the higher values can be explained by the very poor, or very exceptional, growth of a few individuals.

At first glance, the data would seem to indicate that practically all of the rats fed the proteins from fishery products gained more weight than those fed the protein from beef round. This is not true, however, when recognition is given to the effect of variation in food intake (see Tables 4 and 5, p. 5). Food intake was used rather than protein intake to adjust gain in weight. The two com-

ponents are found in exactly the same ratio in all diets, but the absolute quantity of food intake varied more than the quantity of protein intake. It is more likely that variations in gain in weight are due to quality rather than quantity

Table 2 - Mean and Estimated Gain in Weight of Rats Fed for a 10-Week Period with Diets Containing 9 Percent of Protein from Fishery Products

| Source of Protein | Number |         | Mean gain in weight |         |       | Estimated mean gain for group on basis of equal food intake |
|-------------------|--------|---------|---------------------|---------|-------|---|
|                   | Males  | Females | Males               | Females | Group |   |
|                   |        |         | Grams               | Grams   | Grams | Grams   |
| Beef round .....  | 12     | 10      | 136.3               | 121.8   | 129.7 | 145.2   |
| Bonito .....      | 4      | 6       | 166.0               | 141.7   | 151.4 | 141.5   |
| Catfish .....     | 4      | 6       | 190.8               | 117.3   | 146.7 | 150.7   |
| Halibut .....     | 4      | 6       | 144.2               | 136.2   | 139.4 | 147.1   |
| Herring:          |        |         |                     |         |       |   |
| Lake .....        | 7      | 4       | 152.7               | 113.5   | 138.5 | 152.7   |
| Sea .....         | 6      | 4       | 183.7               | 130.3   | 162.3 | 163.2   |
| Mullet .....      | 4      | 6       | 183.5               | 123.5   | 147.5 | 151.3   |
| Salmon:           |        |         |                     |         |       |   |
| Chinook .....     | 6      | 9       | 147.5               | 110.3   | 125.2 | 139.3   |
| Chum .....        | 4      | 6       | 180.3               | 142.7   | 157.7 | 146.0   |
| Coho .....        | 5      | 7       | 135.8               | 123.6   | 128.7 | 140.3   |
| Pink .....        | 4      | 6       | 174.5               | 127.0   | 146.0 | 142.1   |
| Sockeye .....     | 4      | 6       | 170.5               | 130.7   | 146.6 | 150.6   |
| Squeteague .....  | 4      | 6       | 133.3               | 113.7   | 121.5 | 139.9   |
| Trout, lake ..... | 4      | 6       | 177.0               | 126.7   | 146.8 | 133.5   |
| Tuna:             |        |         |                     |         |       |   |
| Albacore .....    | 3      | 7       | 150.0               | 154.0   | 152.8 | 138.2   |
| Bluefin .....     | 4      | 6       | 186.0               | 150.5   | 164.7 | 142.2   |
| Skipjack .....    | 4      | 6       | 154.3               | 149.3   | 151.3 | 134.8   |
| Yellowfin .....   | 4      | 6       | 164.8               | 129.5   | 143.6 | 140.6   |
|                   |        | Mean    | 162.8               | 130.1   | 144.5 | 144.5   |

differences in proteins after eliminating the effect of variations in food intake. The statistical analyses show clearly that there was no difference in the response of the two sexes to the experimental variables.

Table 3 - Statistical Analysis of Mean Gain in Weight of Rats for the 10-Week Period as Recorded in Table 1

| Source of Protein | Coefficient of variation |                  |                  | Source of Protein | Coefficient of variation |         |         |
|-------------------|--------------------------|------------------|------------------|-------------------|--------------------------|---------|---------|
|                   | Males                    | Females          | Group            |                   | Males                    | Females | Group   |
|                   | Percent                  | Percent          | Percent          |                   | Percent                  | Percent | Percent |
| Beef round .....  | 23                       | 16               | 21               | Salmon (Cont.)    |                          |         |         |
| Bonito .....      | 18                       | 7                | 15               | Coho .....        | 20                       | 27      | 24      |
| Catfish .....     | 15                       | 20               | 30 <sup>1/</sup> | Pink .....        | 10                       | 13      | 6       |
| Halibut .....     | 11                       | 15               | 13               | Sockeye .....     | 10                       | 8       | 16      |
| Herring:          |                          |                  |                  | Squeteague .....  | 10                       | 17      | 16      |
| Lake .....        | 17                       | 32 <sup>2/</sup> | 25               | Trout, lake ..... | 24                       | 10      | 25      |
| Sea .....         | 13                       | 6                | 21 <sup>3/</sup> | Tuna:             |                          |         |         |
| Mullet .....      | 18                       | 13               | 26               | Albacore .....    | 32 <sup>4/</sup>         | 16      | 20      |
| Salmon:           |                          |                  |                  | Bluefin .....     | 17                       | 14      | 18      |
| Chinook .....     | 21                       | 20               | 25               | Skipjack .....    | 25                       | 22      | 22      |
| Chum .....        | 14                       | 16               | 19               | Yellowfin .....   | 17                       | 15      | 20      |

<sup>1/</sup>One male grew very well, and two females grew poorly. <sup>3/</sup>Three males grew very well.

<sup>2/</sup>One female grew very poorly.

<sup>4/</sup>One male grew very poorly.

The standard error of difference between adjusted mean group gains in weight equals 28.96 g., and a difference to equal the 5 percent level of significance for 180 degrees of freedom is 57.1 g. (Table 5). It will be noted in Table 2 that the greatest difference in original data for mean gain in weight for groups is 43.2 g. (164.7 - 121.5 g.), and for estimated mean gain in weight it is 29.7 g.

(163.2 - 133.5 g.). This means that all of the proteins studied had about the same nutritive value in these feeding tests.

Table 4 - Mean Food Intake of Rats Fed for a 10-Week Period with Diets Containing 9 Percent of Protein from Fishery Products

| Source of Protein | Mean Food Intake |         |       | Source of Protein | Mean Food Intake |         |       |
|-------------------|------------------|---------|-------|-------------------|------------------|---------|-------|
|                   | Males            | Females | Group |                   | Males            | Females | Group |
|                   | Grams            | Grams   | Grams |                   | Grams            | Grams   | Grams |
| Beef round .....  | 620.6            | 622.6   | 621.5 | Salmon (Cont.):   |                  |         |       |
| Bonito .....      | 721.3            | 731.0   | 727.1 | Coho .....        | 631.4            | 697.8   | 641.0 |
| Catfish .....     | 766.5            | 608.2   | 671.5 | Pink .....        | 739.5            | 679.0   | 703.2 |
| Halibut .....     | 633.0            | 672.0   | 656.4 | Sockeye .....     | 701.8            | 651.2   | 671.4 |
| Herring:          |                  |         |       | Squeteague .....  | 601.5            | 622.0   | 613.8 |
| Lake .....        | 665.3            | 569.8   | 630.5 | Trout, lake ..... | 795.5            | 704.2   | 740.7 |
| Sea .....         | 739.3            | 600.8   | 683.9 | Tuna:             |                  |         |       |
| Mullet .....      | 741.3            | 626.7   | 672.5 | Albacore .....    | 704.7            | 764.0   | 746.2 |
| Salmon:           |                  |         |       | Bluefin .....     | 815.0            | 752.7   | 777.6 |
| Chinook .....     | 660.2            | 612.0   | 631.3 | Skipjack .....    | 729.8            | 769.3   | 753.5 |
| Chum .....        | 775.5            | 706.8   | 734.3 | Yellowfin .....   | 731.5            | 678.3   | 699.6 |
|                   |                  |         |       | Mean .....        | 709.7            | 670.5   | 687.6 |

The data in Table 6 on grams gain in live weight per gram of protein consumed, particularly for group means, show about the same degree of variation and

Table 5 - Analysis of Variance for Food Intake and Gain in Weight

|   | Source of Variation |        |            |
|---|---------------------|--------|------------|
|   | Groups              | Sex    | Residue    |
| Degree of freedom                         | 17                  | 1      | 180        |
| Sum of squares:                           |                     |        |            |
| Gain in weight                            | 29,755              | 44,999 | 74,845     |
| Food intake                               | 498,072             | 45,479 | 1,512,420  |
| Product of gain in weight and food intake | 103,063             | 45,238 | 377,825    |
| Adjusted sums of squares                  | 200,364.56          | 0.72   | 829,935.81 |
| Adjusted mean squares                     | 11,786.15           | 0.72   | 4,610.75   |
| Adjusted standard deviation               | 108.564             | 0.849  | 67.903     |

Standard error of difference =  $\frac{67.903}{\sqrt{11}} \times \sqrt{2} = 28.96$  grams  
 Difference in group mean gain in weight to equal 5 percent level = 57.1 grams  
 Largest difference in original data = 43.2 "  
 Largest difference in estimated data = 29.7 "

Note: Method of Titus and Hammond, 1935.

trend as do the data on adjusted gain in weight as reported in Table 2. The rats fed the protein prepared from sea herring apparently made the most efficient gain

Table 6 - Total Protein Required for Gain in Weight

| Source of Protein | Gain Per Gram Protein |         |       | Source of Protein | Gain Per Gram Protein |         |       |
|-------------------|-----------------------|---------|-------|-------------------|-----------------------|---------|-------|
|                   | Males                 | Females | Group |                   | Males                 | Females | Group |
|                   | Grams                 | Grams   | Grams |                   | Grams                 | Grams   | Grams |
| Beef round .....  | 2.14                  | 1.91    | 2.04  | Salmon (Cont.):   |                       |         |       |
| Bonito .....      | 2.25                  | 1.90    | 2.04  | Coho .....        | 2.10                  | 1.87    | 1.97  |
| Catfish .....     | 2.44                  | 1.88    | 2.10  | Pink .....        | 2.33                  | 1.84    | 2.03  |
| Halibut .....     | 2.26                  | 1.98    | 2.09  | Sockeye .....     | 2.39                  | 1.98    | 2.14  |
| Herring:          |                       |         |       | Squeteague .....  | 2.17                  | 1.79    | 1.94  |
| Lake .....        | 2.24                  | 1.93    | 2.13  | Trout, lake ..... | 2.17                  | 1.76    | 1.93  |
| Sea .....         | 2.43                  | 2.14    | 2.31  | Tuna:             |                       |         |       |
| Mullet .....      | 2.42                  | 1.93    | 2.13  | Albacore .....    | 2.06                  | 1.97    | 2.00  |
| Salmon:           |                       |         |       | Bluefin .....     | 2.23                  | 1.97    | 2.07  |
| Chinook .....     | 2.19                  | 1.77    | 1.94  | Skipjack .....    | 2.07                  | 1.89    | 1.96  |
| Chum .....        | 2.28                  | 1.97    | 2.10  | Yellowfin .....   | 2.20                  | 1.87    | 2.00  |
|                   |                       |         |       | Mean .....        | 2.24                  | 1.91    | 2.05  |

in weight of the series. Three of the male rats grew very well, and the female rats made consistent and uniformly high gains in live weight.

The apparent digestibility of the total protein in the diet was determined by collecting feces during one week of the experimental period for rats selected at random (Table 7). Paper towels were spread under the cages and the feces were collected every day or two during the interval of the fourth to tenth week on experiment, and there was no evidence that the apparent digestibility varied with age of rat.

Table 7 - The Apparent Digestibility of Total Protein for Individual Rats During a 1 Week Period

| Source of Protein | APPARENT DIGESTIBILITY IN PERCENT |       |       |       |       |       |       |       |       |      |  | Mean |
|-------------------|-----------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|------|--|------|
|                   | Individual rats                   |       |       |       |       |       |       |       |       |      |  |      |
| Beef round .....  | 90.2,                             | 86.1, | 86.7, | 90.5, | 88.1, | 93.2, | 86.4, | 87.5, | 87.7, | 88.3 |  | 88.5 |
| Bonito .....      | 89.9,                             | 89.4  |       |       |       |       |       |       |       |      |  | 89.7 |
| Catfish .....     | 91.0,                             | 87.8, | 93.3, | 90.3  |       |       |       |       |       |      |  | 90.6 |
| Halibut .....     | 87.4,                             | 86.5, | 89.7, | 90.6  |       |       |       |       |       |      |  | 88.6 |
| Herring:          |                                   |       |       |       |       |       |       |       |       |      |  |      |
| Lake .....        | 91.1,                             | 96.5, | 97.8, | 98.0, | 97.4  |       |       |       |       |      |  | 96.2 |
| Sea .....         | 89.0,                             | 91.6, | 88.2, | 89.4  |       |       |       |       |       |      |  | 89.6 |
| Mullet .....      | 88.7,                             | 87.8, | 88.3, | 86.1  |       |       |       |       |       |      |  | 87.7 |
| Salmon:           |                                   |       |       |       |       |       |       |       |       |      |  |      |
| Chinook .....     | 89.4,                             | 90.9, | 92.5, | 92.4  |       |       |       |       |       |      |  | 91.3 |
| Chum .....        | 88.4,                             | 89.2, | 88.4  |       |       |       |       |       |       |      |  | 88.7 |
| Coho .....        | 90.8,                             | 89.1, | 90.7  |       |       |       |       |       |       |      |  | 90.2 |
| Pink .....        | 89.2,                             | 85.2, | 90.7  |       |       |       |       |       |       |      |  | 88.4 |
| Sockeye .....     | 87.6,                             | 87.3, | 91.3  |       |       |       |       |       |       |      |  | 88.7 |
| Squeteague .....  | 92.0,                             | 92.3, | 90.4  |       |       |       |       |       |       |      |  | 91.6 |
| Trout, lake ..... | 88.7,                             | 90.9, | 87.9, | 89.6, | 90.9  |       |       |       |       |      |  | 89.6 |
| Tuna:             |                                   |       |       |       |       |       |       |       |       |      |  |      |
| Albacore .....    | 89.5,                             | 90.5, | 90.2, | 89.9  |       |       |       |       |       |      |  | 90.0 |
| Bluefin .....     | 89.0,                             | 90.7  |       |       |       |       |       |       |       |      |  | 89.9 |
| Skipjack .....    | 88.7,                             | 87.1, | 89.3  |       |       |       |       |       |       |      |  | 88.4 |
| Yellowfin .....   | 90.1,                             | 91.5, | 90.4  |       |       |       |       |       |       |      |  | 90.7 |
|                   |                                   |       |       |       |       |       |       |       |       |      |  | 89.9 |

Very little variation in apparent digestibility is noted. The highest mean value is 7 percent, and the lowest value is only 2.4 percent under a "mean" apparent digestibility value for all groups. All of the proteins were well digested.

## DISCUSSION

The experimental data indicate that the 18 proteins fed were about equally valuable in promoting growth at the 9 percent level in the diet. Lanham and Lemon (1938), on the other hand, found a difference in the nutritive value of the proteins which they fed at the same level, and concluded that those of fishery products were superior to that of beef round by 1.3 to 1.6 times. It will be noted that the rats used in their tests did not grow as well as those used in this study. The 12 rats of their control group fed beef, gained an average of 95.2 g. during the 10-week period as compared with 22 rats of the present series gaining an average of 129.7 g. Eleven rats fed protein from coho salmon gained 104.4 g. compared with 12 rats gaining an average of 128.7 g. in this study.

There are several possible explanations for these differences. First, the rats used in the earlier investigation were housed in a building that did not have very efficient control of temperature, and they may have been chilled or too warm at times. An environmental temperature of 80° F. was maintained during the test

period for the experiments reported herein. Secondly, the vitamin supplementation consisting of a commercial vitamin concentrate from milk, and an alcoholic extract of wheat embryo may not have been as complete in quantity or quality as the combination used in this instance. Also, the basal diet used previously contained 0.68 percent protein as compared with 1.2 percent protein in the latter series of experiments.

It is unlikely that the protein from the small quantity of liver extract, wheat embryo, and yeast in the diet would be responsible for the non-significant differences in gain in weight found in these data. There are appreciable but not statistically significant differences between groups which can most logically be attributed to varying quality of the fishery proteins. These differences may have been greater if the diet contained no supplementary protein. The supplementary proteins undoubtedly exert some balancing effect, but probably no more than that furnished by the protein of the average American diet. The data do show that the proteins of the fishery products tested are at least equal to beef in nutritive value.

### SUMMARY

1. The proteins from 17 species of fish which were tested were found to be about equal in nutritive value when included in a diet containing some supplementary protein.

2. The nutritive value of these proteins was found to be about equal to that of the protein of beef.

3. The proteins were well digested, having an apparent digestibility of about 90 percent.

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