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A METHOD FOR EVALUATION OF THE NUTRITIVE VALUE OF A PROTEIN

A Comparison of Broiled and Pan-fried Striped Bass Fillets

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INTRODUCTION

Dietitians and nutritionists have more recently shown considerable interest in the effect of cooking on the nutritive value of various foods. Almost all of the early assays of nutrient elements in foods was limited to raw foods. This work is valuable in order to determine the expected variability in the amounts of nutrient elements in the raw product, but does not permit the evaluation of a serving portion in terms of the recommended daily allowances of the various nutrient elements. More emphasis should now be placed on determinations of the nutritive values of the foods as served.

Marks and Nilson (1946) reported that baking, broiling, boiling, or simmering had no adverse effect on the nutritive value of the protein of cod. Martinek and Goldbeck (1947) reported that baking at 375° and 500° F. had no differential effect on the nutritive value of the protein of croaker fillets. The methods of cooking which were chosen for these studies did not require the addition of any other products, except a light brushing of oil on the baked or broiled fish.

So far no results have been reported for pan-fried (sauteed) fish although pan-frying is the usual method of preparation. This method, however, requires that the serving portion be coated with a cereal or a mixture of cereals and binders, and be cooked in a pan containing a small amount of foreign fat. From the experimental standpoint, the number of uncontrollable variables of composition is increased, but none which is probably of great significance.

Since the nutritive value of the protein in foodstuffs cannot be determined with any degree of accuracy by chemical analyses, resort must usually be had to experimental feeding of animals. A widely used method is that promulgated by Osborne, Mendel and Ferry (1919). This method recommends that weanling rats be fed ad libitum a basal diet free from protein, but otherwise nutritionally complete. To this diet is usually added the equivalent of ten percent of protein in the form of the dried foodstuff to be tested. The biological value of the protein for growth is calculated as the ratio of grams gain in liveweight to the grams of protein ingested during the test period.

One objection to this method is that differences in gains in liveweight may not be due solely to differences in the quantity and quality of protein. Neither is any correction usually made for differences in intake of calories. Also, the vitamin content of the diet ordinarily used may not permit maximum growth in respect to protein intake. It is apparently not yet possible to formulate a basal diet containing all essential accessory growth factors except through the inclusion of some nitrogen-containing concentrate. This is particularly true in the assay of foods containing a fairly high level of protein and a low concentration of vitamins.

The criticisms are valid. It is possible to minimize the effect of variable food intake by limiting daily the food intake of a pair of rats to that quantity which one member of the pair consumes voluntarily. This method is time consuming to carry out, and is of no advantage unless both animals of a pair have a high degree of similarity genetically, and unless either animal has about an equal chance of determining the daily food intake. Another method involves evaluation by statistical methods of the effect of variable food intake (Crampton and Hopkins, 1934). This method seemingly offers good possibilities, at least under certain circumstances, for the establishment of the significance of differences between groups when gain in liveweight is used as the criterion of the quality of protein. Unpublished data from our laboratory indicate that the statistical correction of group data is not feasible unless the animals are allotted to the various groups with about equal distribution of litter mates and sex, and unless the diets are reasonably similar in respect to quantity of protein consumed (Nilson and Martinek, 1945, and Marks and Nilson, 1946). The degree of permissible variability of these factors has not been established.

It is admitted that a true index of the nutritive quality of a single protein cannot be established when one or more vitamin concentrates containing protein are used to insure maximum growth within the limitation imposed by the intake of test protein. Actually, the balancing or supplementary value of the test protein is determined. The establishment of this index is valuable from the standpoint of every day nutrition because it permits evaluation of the nutritive quality of a protein in respect to the daily diet, on the assumption that the protein available in the average diet is equal in balancing value to that of the protein of the vitamin concentrates used in the experimental diet. This requirement should be fulfilled if the daily diet contains a reasonable amount of animal protein.

A potentially serious objection to assays depending on statistics of growth is that the gain in liveweight of one group of animals may represent a different nutritive investment than the gain in liveweight of a second group of animals. In one case more protein may be stored, and less may be used for energy. Fat and water may be stored in different proportions. Ordinarily, this contingency is guarded against by careful allotment of animals, by keeping as small as possible the variations in the nutritive values of the diet and by conducting the experiments over a fairly short span of the active growth period.

Another fundamental method for the determination of the nutritive value of a protein is that proposed by Mitchell (1924 and 1943). It has the advantage of being of short duration so that the nutritive balance of the basal diet is of little importance. This method is based on measurement of the output of nitrogen in the urine and feces during a three or more day period when the rat is fed either a nitrogen free diet, or one containing a small amount of a very high quality of protein which is considered to be completely digested and utilized. During the second collection period which takes place within a few days of the first, the rats are fed a quantity of test protein equivalent to the nitrogen contained in the urine during the first period. A third collection period is similar to the first, and the data obtained may be combined with that obtained during the first period to determine an average value. Adult male rats are usually used, so the index established is the biological value for maintenance.

The biological value is expressed by the formula: 100 times the ratio of food nitrogen minus (fecal nitrogen during protein feeding period minus fecal nitrogen during basal feeding period) minus (urinary nitrogen during protein feeding period minus urinary nitrogen during basal feeding period) divided by food nitrogen minus (fecal nitrogen during protein feeding period minus fecal nitrogen during basal feeding period). The maximum biological value equals 100.

The method is fundamentally sound, but it is difficult to carry out satisfactorily. The rats often do not consume enough basal diet to maintain weight, so they have to be discarded. Sometimes a small quantity of a very high-quality protein must be incorporated in the basal diet to make it more palatable and thus insure satisfactory consumption. It is also difficult in many instances to measure accurately the protein intake even though the protein test sample is incorporated in a sugar and agar gel. Pieces often drop through the screen floor and cannot be accurately separated from the feces or urine samples. The urine samples are reasonably satisfactory although there is an unknown decomposition loss in the usual method of collection. Blood and semen are often contaminants, and some urine is absorbed by the feces and loose hair over which it is spilled. The feces samples are less satisfactory, since they are always contaminated by comparatively large quantities of hair which the animal has swallowed in cleansing itself. The feces collected during the protein feeding period are likely to be hard in texture and difficult to grind. Also a large number of Kjeldahl determinations must be made.

The method is very useful in spite of the enumerated limitations and the seemingly unreasonable values which are too often obtained. It is true, of course, that such values are also gotten occasionally for individual rats during growth tests. Both methods permit evaluation of only the crude protein in the sample. No separation can be made of the effect of the true protein and the nonprotein nitrogenous matter. This limitation is of minor importance in respect to most fishery products used for human consumption, since nearly all of the nitrogen is in the protein.

The object of the following reported experiments was to determine the nutritive value of the proteins of broiled and pan-fried striped bass fillets by use of the modified Osborne, et al method previously employed in this laboratory. This method differs from the original in that the test protein is fed daily in equal amounts to individual rats and in such quantity that there is a relatively constant ratio between the intake of the protein supplement fed separately, and the basal diet consumed when offered ad libitum. A statistical method using multiple regressions is employed to adjust or estimate the gain in liveweight for theoretically equal intake of calories and other variable factors based on the data for all animals (Snedecar, 1940, see table 3). The statistical significance of differences between groups or sub-groups can then be determined.

EXPERIMENTAL METHODS AND DATA

Feeding Studies. - Fresh striped bass (Roccus saxatilis) were purchased at the local market and filleted with skins left on. Half of the fillets were brushed lightly with cottonseed oil and placed in a preheated broiler at a moderate heat (350° to 375° F.). Broiling was completed in about 20 minutes when the fillets were brown on both sides after being turned. The remaining half of the fillets were dipped in water, and covered lightly with cornmeal and were pan-fried in a small quantity of cottonseed oil. Each of the two lots of cooked fillets were then ground twice through an electric food chopper, and formed into blocks. These were wrapped in moisture-vaporproof cellophane, quick frozen, and stored at a low temperature until used. The proximate analyses of samples used indicate that there was no important difference in the protein content of the broiled and fried fillets. There was about a five percent greater fat content in the broiled fillets with about a corresponding decrease in moisture content (Table 1).

Ten albino rats, weighing 49 to 55 grams each were allotted to each of the two test groups. The rats were housed individually in wire screen cages fitted with wire screen floors. An environmental temperature of about 80° F. was maintained. Water and the basal diet were available at all times. The experiment lasted eight weeks.

The basal diet consisted of corn starch, 80; lard, 10; cod liver oil, 2; salt mixture No. 2, U. S. P. XI, 4; wheat embryo, 2; dried brewer's yeast, 1.5; and liver extract, Lilly, 0.5 parts by weight. This diet contained 1.6 percent protein. During the first and second weeks each rat received an equivalent of 0.60 gram of supplementary protein each day. This was increased to 0.85 gram during the third, fourth, and fifth weeks, and 1.20 grams during the remainder of the experimental period. It will be noted that according to the data in Table 1, the pan-fried fillets had a higher caloric value than the broiled fillets. This was not corrected for in the food intake data since calculations

Table 1. Proximate analyses of samples used.

Sample of fillet	Percent by Weight: Dry Matter	Protein (NX6.25)	Ether extract	Mineral matter (ash)
Lot 1:				
Raw	23.80	20.90	1.32	1.38
Broiled	32.10	24.50	5.93	1.52
Pan-fried	40.40	24.05	10.70	1.46
Lot 2:				
Broiled	30.98	23.88	6.21	1.49
Pan-fried	37.36	22.79	10.88	1.30

showed that several pairs of rats, consuming within a gram or so of equal quantities of the two types of fillets and basal diet, had calculated calorie intakes which differed only by about three percent during the eight week period.

The data in Table 2 show that the mean gain in liveweight was 120.3 grams for those fed broiled fillets and 114.6 grams for those fed fried fillets. The difference was only 5.7 grams. When an adjustment was made in the mean gain in liveweight to correct for differences in food consumption, namely 56.20 grams protein and 459.5 grams food versus 56.34 grams protein and 433.0 grams food respectively, the estimated weight differed by only a fraction of a gram from the actual weight for each group (Table 2 and 3). The mean error of estimate between actual and estimated gains in liveweight of 0.5 gram (-0.3 + 0.2 gram) when divided by the standard error of mean difference of 4.92 indicates a non-significant difference. In other words, the protein of broiled and pan-fried fillets is of equal nutritive value.

If the index recommended by Osborne, et al for the nutritive value of protein is used, the rats fed broiled striped bass increased in liveweight 2.14 grams (standard error, 0.1040 gram) per gram of protein consumed and those fed the fried fish gained 2.03 grams (standard error, 0.0643 gram) (Table 2). The difference between the two means is not statistically significant according to the conventional "t" test. Similarly, there is not a statistically significant difference between groups for the indexes of efficiency commonly used in farm-animal feeding experiments, namely, weight of food consumed per unit gain in liveweight. When the data of individual rats were used it was found that those fed the broiled fillets required 3.891 grams (standard error, 0.1902 gram) and those fed fried fillets needed 3.798 grams (standard error, 0.1002 gram) of air dried food per gram gain in liveweight. These findings confirm the conclusion arrived at earlier that there was no difference in the effect of the two cooking methods on the nutritive value of the protein.

Incidentally, Darling (1946) found that a group of four rats fed a diet similar to that used in the experiment reported herein, but with roast beef as the protein being tested increased 115.3 grams in liveweight over an eight week period. The protein consumption was 55.32 grams, and the food intake was 406.0 grams. These data are not very different from those reported for the rats fed striped bass. It may be concluded from a comparison of data from the two experiments that the nutritive value of the protein of striped bass fillets which have been broiled or pan-fried is about equal in nutritive value to that of beef.

From the standpoint of experimental methods, several interesting questions remained to be answered. When the rats were divided into sub-groups according to sex and type of fish fed, it was not possible to increase materially the values of the correlation coefficients between the protein intake or the total food intake, and the gain in liveweight. It was, therefore, thought advisable to present the data for individual rats (Table 2). These data show that the actual gains in liveweight ranged from 98 to 154 grams for those fed broiled fillets and from 104 to 147 grams for those fed fried fillets. The respective estimated gains in liveweight, based on the data for the two groups as a single unit, ranged from 109 to 130 grams for the rats fed broiled fillets and 104 to 126 grams for the rats fed the fried fillets. Only three rats out of the 20 showed a difference in excess of 15 grams between actual and estimated gains.

Table 2: Data on the gain in liveweight and food intake for groups, and for individual rats fed broiled or pan-fried fillets of striped bass for an eight week period.

Diet designation and rat number	Number of rats	Protein in diet as consumed	Gain in liveweight actual	Gain in liveweight estimated	Total protein intake	Food intake 1/	Gain in liveweight per gram protein intake actual	Gain in liveweight per gram protein intake estimated	Food intake per gram gain in liveweight actual	Food intake per gram gain in liveweight estimated 2/
		percent	grams	grams	grams		grams	grams	grams	grams
For groups fed:										
Broiled fillets	10	12.2	120.3	120.6	56.20	459.5	2.14	2.15	3.82	3.81
Fried fillets	10	13.0	114.6	114.4	56.34	433.0	2.03	2.03	3.78	3.79
Overall mean		12.6	117.5		56.27	446.3	2.09		3.80	
Individual rats fed:										
Broiled fillets										
Males:										
5 3/		11.67	133	126.3	56.61	485	2.35	2.23	3.65	3.84
12		11.86	136	124.3	56.46	476	2.41	2.20	3.50	3.83
14		11.97	154	123.2	56.38	471	2.73	2.19	3.06	3.82
19		11.69	136	126.1	56.59	484	2.40	2.23	3.56	3.84
	4	11-12	140	125.0	56.51	479	2.48	2.21	3.42	3.83
7	1	12.31	123	119.8	56.14	456	2.19	2.13	3.71	3.81
Females:										
9	1	11.29	99	130.6	56.91	504	1.74	2.29	5.09	3.86
1		12.80	109	115.4	55.82	436	1.95	2.07	4.00	3.78
11		12.43	104	118.7	56.06	451	1.86	2.12	4.34	3.80
	2	12-13	107	117.2	55.94	444	1.91	2.10	4.15	3.79
3		13.15	98	112.4	55.62	423	1.76	2.02	4.32	3.76
16		13.54	111	109.3	55.39	409	2.00	1.97	3.68	3.74
	2	13 +	105	110.9	55.51	416	1.81	2.00	3.96	3.75
Fried fillets										
Males:										
18	1	11.77	147	126.3	57.18	486	2.57	2.21	3.31	3.85
6	1	12.80	113	116.2	56.46	441	2.00	2.06	3.90	3.80
10	1	13.50	105	110.3	56.04	415	1.87	1.97	3.95	3.76
Females:										
8		12.80	104	116.2	56.46	441	1.84	2.06	4.24	3.80
13		12.27	116	121.1	56.81	463	2.04	2.13	3.99	3.82
20		12.00	114	123.8	57.00	475	2.00	2.17	4.17	3.84
	3	12-13	111	120.4	56.76	460	1.96	2.12	4.14	3.82
2		13.28	112	112.1	56.17	423	1.99	2.00	3.78	3.77
4		14.36	116	104.0	55.59	387	2.09	1.87	3.34	3.72
15		14.04	107	106.3	55.75	397	1.92	1.91	3.71	3.73
17		13.89	112	107.4	55.83	402	2.01	1.92	3.59	3.74
	4	13 +	112	107.4	55.84	402	2.01	1.92	3.59	3.74

1/ Basal diet intake, plus dry matter of fish increased by 15 percent. This was done to approximate air dry equivalent of basal diet.

2/ According to Snedecor, 1940.

3/ Sub-groups are coded from the individual rats which consumed diets containing less than 12, 12 to 13, and more than 13 percent protein in the diet.

Table 3: Calculations of sums of squares and products, and correlation coefficients of data for groups presented in Table 1.

$n = 20$	Protein intake X_1	Food intake X_2	Gain in liveweight Y
Sums	1,125.3	8,925	2,349
Means	56.27	446.3	117.5
SX_1^2, SX_1X_2, SX_1Y	63,331.2	502,489.9	132,233.1
Correction term	63,315.005	502,165.13	132,166.49
sx_1^2, sx_1x_2, sx_1y	16.195	324.77	66.61
$\sqrt{sx_1^2}$, $\sqrt{(sx_1^2)(sx_2^2)}$, etc.	4.0243	597.7643	276.5648
r^{12}, ry^1		0.5433	0.2408
SX_2^2, SX_2Y		4,004,845.0	1,053,207.0
Correction term		3,982,781.25	1,048,241.25
sx_2^2, sx_2y		22,063.75	4,965.75
$\sqrt{sx_2^2}$, $\sqrt{(sx_2^2)(sy^2)}$		148.5387	10,208.1291
ry^2			0.4865
SY^2			280,613.0
Correction term			275,890.05
sy^2			4,722.95
$\sqrt{sy^2}$			68.7237
sy			15.7663

The regression equation is $\hat{Y} = 45.39 - 0.5704X_1 + 0.2335X_2$

R equals 0.2374

Two of these were male rats which each gained 21 grams more than an estimate of food consumption would warrant. One of these males was allotted to each of the two groups. The third rat, a female fed the broiled fillets, gained only about 3/4 as much as would be expected from the food consumption. This rat consumed comparatively large amounts of basal diet during each week of the experiment. Apparently, the basal diet was not utilized very efficiently.

The data indicate that the basal diet was consumed in relation to the intake of protein supplement. The percent protein in the diet as consumed (air-dried basis) ranged from 11.29 to 13.54 for the rats fed broiled fillets, and from 11.77 to 14.36 for the rats fed fried fillets. The males as a general rule consumed more basal diet than the females, but the increased amount cannot account for the differences in gain in liveweight. The extra gain may rather be due to a more efficient utilization of protein for growth by most males and to a greater need of protein by the females for certain physiological functions. Gain in liveweight under these experimental conditions was limited principally by protein intake, and to a very secondary extent by intake of basal diet even though the latter was offered ad libitum.

Carcass Analyses. - In the introduction of this paper it was stated that a potentially serious objection of the growth method for determination of the nutritive quality of a protein is that the gain in liveweight of one group of animals may represent a different nutritive investment than that of another group of animals. In other words, the various groups which are being compared may have a significantly different store of protein, fat, mineral matter, or water. In order to determine what happened in the herein reported experiment, four pre-experimental rats and six post-experimental rats from each of the two groups were killed, and the contents of the gastro-intestinal tract were removed by pressure stripping. The carcasses were frozen pending analyses. The frozen carcasses were chopped lengthwise, and the half carcass was ground through a meat chopper. After the ground samples were thoroughly mixed, samples of appropriate size were analyzed by standard methods to determine the content of moisture, crude protein, ether extract, and mineral matter. The other half of the carcass was reserved for check analyses. Five check analyses for protein and six for moisture were made by another chemist and gave reasonably good agreement with the original results.

The data in Table 4 on proximate analyses expressed in percent by weight do not indicate any outstanding differences between individuals of the two groups.

The data in Table 5 also show no outstanding differences between the two groups. Statistically non-significant differences were found between group means for the empty carcass weight and the store of protein, ether extract and mineral matter. There was, however, a statistically significantly greater storage of protein by the male rats fed the broiled fillets than by the females. This sex difference was not apparent for the rats fed the fried fillets. Why this happened cannot be ascertained from these studies. The correlation coefficients between the intake and storage of the designated nutrient were essentially non-significant. For protein, the coefficients were 0.3740 and 0.3939, for ether extract, -0.2829 and -0.1956, and for mineral matter 0.4120 and 0.5935, respectively, for the animals fed the broiled and the fried fillets.

Table 4: Proximate analyses of the empty carcasses.

Diet designation and rat number	Sex	Percent by weight: Moisture	Protein (NX6.25)	Ether extract	Mineral matter
Pre-experimental:					
	M	70.8	15.3	8.9	2.8
	M	68.5	16.0	10.5	2.9
	F	72.1	18.8	5.6	3.0
	F	71.5	18.3	7.1	3.8
After eight weeks: Broiled fillets					
5	M	64.3	17.2	13.8	2.7
14	M	61.9	17.4	15.3	3.6
19	M	61.1	17.9	16.5	3.2
1	F	62.4	17.3	16.0	2.9
3	F	62.4	17.8	15.4	2.9
9	F	64.8	18.0	11.7	3.4
Pan-fried fillets					
6	M	65.6	18.5	10.7	3.4
10	M	67.4	18.3	8.9	3.2
2	F	59.8	20.7	14.1	3.4
8	F	61.2	18.5	15.1	3.1
15	F	62.9	17.6	15.2	3.0
17	F	64.4	17.4	13.4	2.8

The differences between groups in mean protein and total food intake were not statistically significant. The rats fed the broiled fillets, however, had a mean intake of mineral matter of 21.8 grams as compared with 19.6 grams for those fed fried fillets. The difference was statistically significant. The ratios of intake to storage of mineral matter tend to reflect this difference.

The approximate ratios of intake to storage show that about 37 percent of the crude protein was stored. The range was from 30 to 47 percent. This indicates that a good deal of the protein was utilized for physiological purposes not associated with permanent tissue structure or as a source of energy. Recalculation of the data for storage of fat (ether extract), indicates that about 11 percent of the total caloric intake was stored as fat. The range was roughly from 5 to 12 percent. About 17 percent of the total caloric intake was stored as combined protein and fat. The range was from 10 to 18 percent.

All rats reacted within reasonably close limits in respect to storage of protein. This was probably because protein was the limiting nutrient in the diet. Nine of the twelve rats stored about an equal proportion of the calories consumed. Female number 9 and males number 6 and 10 stored less fat in the body which means that they were more wasteful in using calories for physiological purposes.

DISCUSSION

The two groups of rats fed the broiled and the pan-fried (sauteed) striped bass fillets grew at a very similar rate when fed equal quantities of the test protein. According to unpublished data from our laboratory, the gain in live-weight was only about one-half that of the rats which had been fed for an equal length of time on a control diet which contained about twice as much protein of high nutritive quality and which was otherwise balanced for essential nutritive elements. Inherent genetic capacity for growth was probably the only limiting factor in this instance, since environmental factors were about optimum.

In the feeding experiments to determine the nutritive value of the proteins of broiled and pan-fried striped bass fillets, the factors that limited growth were the quantity of available protein, the quality of the protein, and the quantity of total food intake as representing qualitatively sufficient calories, vitamins, and mineral elements to permit maximum growth.

Quantity of protein in this instance was the primary factor limiting growth. It was the fixed variable in the diet, limiting not only growth but also the consumption of the basal diet which was offered *ad libitum.* Furthermore, the quantity of test protein available to the animal was limited physically by the quantity fed and eaten, and by the quantity digested by the animal. The physical quantity was limited to about 50 grams per rat during the eight week period and was all consumed. The quantity digested was not determined.

Previous studies by Lanham and Lemon (1938) and Nilson and Martinek (1946) had shown that the apparent digestibility of the protein of somewhat similar diets containing fish protein was about 90 percent. It seems improbable that there was any significant difference in the digestibility of the proteins of the broiled and the fried fillets since the rats receiving the different forms grew at an equal rate during the eight week period.

The quality of protein as affecting gain in liveweight was not an important variable in this study. The nutritive quality of the test proteins must have been equal since the mean estimated gains in liveweight were so similar to the actual gain for both groups.

The quantity of total food intake varied somewhat, but was a minor factor in causing differences in gain in liveweight. This is so because the amount of basal diet eaten was limited within quite narrow limits by the amount of the protein consumed. The maximum range in percent protein in the diet consumed (air-dried basis) was 11.29 to 14.36. Just what factors conditioned the consumption of basal diet cannot be determined from these studies, but caloric need was probably more important than differential vitamin or mineral element requirements. Nine of twelve rats utilised calories within rather narrow limits in storage of fat. Female number 9, one of the exceptions, must have utilized the basal diet very inefficiently (Tables 2 and 5). Just why males number 6 and 10 stored only from 1/3 to 1/2 the quantity of fat stored by the other males cannot be determined from the data. The economy of calorie use must have followed some rather definite physiological pattern when so large a proportion of the animals reacted alike.

The correlation coefficients for the effect of variable protein intake (minor variations were determined entirely by differential intake of the basal diet) on the gain in liveweight is not statistically significant, but that for the effect of the total food intake on the gain in liveweight is statistically significant (Table 3). These findings are in accord with data previously interpreted. The effect of the quantity of test protein fed can be measured only by the feeding of multiple levels. This was not necessary since the quantity fed was less than that permitting maximum growth but it was sufficient to maintain satisfactory health. There is no evidence that the maximum growth permitted by genetic capacity was reached.

The quantity of protein stored in the body varied within narrow limits when expressed as percent by weight of empty carcass or as a ratio of the protein intake. These findings further indicate that the quantity of protein limited growth and that the two test proteins were of equal nutritive value.

The evidence from both the feeding studies and the carcass analyses indicates in general, that the statistically non-significant differences between the groups of rats fed the broiled and the fried striped bass fillets were due to the inherent physiological response of the rats to the diet, rather than that there was so much variability in response of individual rats that any positive difference due to diet was impossible to detect. The coefficient of variation in percent for mean group gains in liveweight was 15.7 for the rats fed the

Table 5: Storage of some of the food elements in the bodies of individual rats during the eight week feeding test with striped bass.

Diet designation and rat number	Sex	Liveweight	Empty 1/ carcass weight	Storage: Protein (NX6.25)	Storage: Ether extract	Storage: Mineral matter	Storage: Remainder by substraction	Approximate ratio of intake to storage: Protein / Protein	Food Ether extract / Ether extract	Mineral matter / Mineral matter
		grams	grams	grams	grams	grams	grams			
Pre-experimental:				In carcass						
	M	53	51	7.96	4.63	1.46	37.0			
	M	54	52	8.16	5.36	1.48	37.0			
	F	51	47	8.84	2.63	1.41	34.1			
	F	51	47	8.60	3.34	1.94	34.1			
Broiled:				In gain in empty carcass weight						
5	M	184	178	22.96	19.81	3.41	82.8	2.5	24.5	6.7
14	M	205	197	26.62	25.39	5.09	90.3	2.1	18.6	3.9
19	M	189	179	24.06	24.59	4.27	75.1	2.4	19.7	5.3
1	F	162	158	18.14	22.11	2.80	65.2	3.1	19.7	7.3
3	F	153	149	17.16	19.72	2.51	58.9	3.2	21.5	7.8
9	F	148	143	17.42	13.86	3.25	63.3	3.3	36.4	7.3
Pan-Fried:										
6	M	162	155	21.26	11.99	3.92	70.7	2.7	36.8	5.3
10	M	155	150	19.95	8.70	3.43	69.8	2.8	47.7	5.6
2	F	166	161	24.14	19.53	3.69	63.9	2.3	21.7	5.3
8	F	154	150	19.25	19.72	3.00	61.9	2.9	22.4	6.0
15	F	156	150	18.08	19.93	2.89	63.9	3.1	19.9	6.4
17	F	163	158	18.82	18.18	2.74	71.3	3.0	22.1	6.8

1/ The ventral surface of the rat was opened carefully to prevent loss of blood, and the gastrointestinal tract was cut loos at esophagus and anus. The tract was emptied by being squeezed between fingers.

broiled fillets and 10.6 for those fed the fried fillets. The coefficients indicate that the variability in gain in liveweight was within satisfactory limits.

No attempt is made to devise or assign any numerical index to represent the nutritive value of the protein. As stated before, the nutritive value of a single protein was not measured, but rather the balancing or supplementary value of the test protein in the diet. Furthermore, there are rather serious objections to the assignment of numerical values to express the nutritive value of a protein. The biological value as an index of nutritive quality is confused by many with the percent digestibility of the protein or protein-containing food. The grams gain in liveweight per gram of protein consumed as an index is appreciated in a comparative way only by the person who is acquainted with experimental work with animals. Any numerical value is likely to be misinterpreted also as to the degree of precision attained. It seems, therefore, to be the better practice to make only non-numerical comparisons.

SUMMARY AND CONCLUSIONS

A method has been proposed which will permit evaluation of the nutritive value of a protein by means of a modified Osborne and Mendel growth method. It requires that approximately equal quantities of test or comparison proteins be consumed by all animals during the experimental period. The quantity of protein to be fed is limited to that which will not permit a normal growth rate but will permit good health. The basal diet consisting of sources of calories, vitamins and minerals is allowed ad libitum.

Estimated gains in liveweight for individual animals are calculated by means of multiple regression to eliminate the effects of the more important variables such as differential sex allotment, caloric intake, etc. The comparative nutritive values of two or more proteins are determined from the differences in estimated group gains in weight.

In this study it was found that the proteins of broiled and pan-fried (sauteed) striped bass fillets possess equal nutritive quality in respect to balancing or supplemental value.

About 37 percent of the protein intake was found to be stored in the body of the rat during the eight-week period. Nine of 12 rats stored a mean of 11 percent of the total caloric intake as fat. They stored about 17 percent of the total caloric intake as combined fat and protein.

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