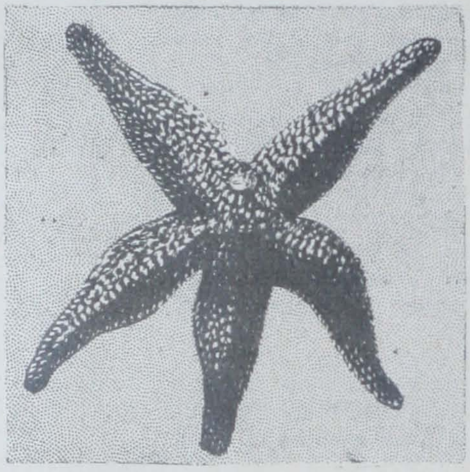
# TECHNOLOGICAL STUDIES OF THE STARFISH

## PART IV—THIAMINASE IN STARFISH

By Charles F. Lee*

## INTRODUCTION

The first two papers of the series of technological studies on the common starfish (Asterias forbesi) of the Atlantic Coastal waters, discussed its ecological relation to the oyster and review data on the chemical composition of fresh starfish and starfish meal. The third paper discussed data from feeding tests of starfish meal as a protein supplement for growth of rats and chicks and for egg production.



The present paper presents the data of the biological tests from which it was concluded that the starfish contained thiaminase, the thiamine-destructive enzyme.

The presence of thiaminase in starfish has already been mentioned in connection with the protein feeding tests reported in the preceding paper of this series, as a factor affecting growth of certain groups of chicks (Lee, 1948-B). It has also been reported in a review paper on thiaminase (Lee, 1948-A). This review summarized information in the literature on the properties of thiaminase, its distribution in the different organs and the various species of fish, and also presented a critical discussion of the numerous contradictions regarding the enzyme that are to be found in the literature. No general discussion of thiaminase will be included herein, other than that which seems to be demanded by the present study of thiaminase in starfish.

## REVIEW OF LITERATURE

The existence of a substance in fish which actively destroyed thiamine was first proven in 1941, although several years earlier, an investigation of a number of outbreaks of paralysis of foxes had traced the cause to a thiamine deficiency, and implicated fish as the dietary factor responsible for it.

It was eventually concluded that the destructive substance was an enzyme, heat labile at the temperature of boiling water, and separating on dialysis into

* Chemical Engineer, Fishery Technological Laboratory, Branch of Commercial Fisheries, College Park, Maryland.

NOTE: Part I of this series, "Starfish Control--Its Economic Necessity and Methods Used," appeared in the January 1948 issue of Commercial Fisheries Review, pp. 1-6. Also available as Sep. No. 193.

Part II, "Chemical Composition," appeared in the February 1948 issue, pp. 11-18. Also available as Sep. No. 196.

Part III, "Value of Starfish Meal--Protein Supplement for Growth of Rats and Chicks and for Egg Production," appeared in the March 1948 issue, pp. 8-19. Also available as Sep. No. 199.

two components of different destructive activity. It is perhaps more accurate to state that the enzyme renders thiamine biologically inactive or unavailable, rather than to refer to its destruction. One process of destruction has been demonstrated to be a hydrolytic splitting of the molecule into its two heterocyclic components, generally referred to as the thiazole and pyrimidine fractions.

It is interesting to note that thiaminase has chiefly been found in what may be broadly called "aquatic animals." All early reports recorded its presence only in fresh-water fish, with the exception of the Atlantic herring. In fish, the greatest concentrations of thiaminase are in the viscera, but it is also widely distributed in the head, skin, and other parts of the fish. There is some controversy as to whether thiaminase is present in the flesh when present elsewhere in the body.

Carp, smelt, and herring are the most important species listed as containing the enzyme, with some 10 or 12 less valuable species; such as, suckers, chubs, burbot, and catfish also containing it. Generally speaking, only a few limited investigations for the determination of the presence of thiaminase have been made of fresh-water species and more especially of the marine species of fish.

Since 1944, thiaminase has been found in a few other strictly marine "animals"; namely, the hard clam, the ocean or black quahog, and the edible mussel. The menhaden has joined the herring as the only true fish in salt water known to contain it.

The occurrence of thiaminase in starfish adds an entirely new phylum of aquatic animals to the list of those which contain the enzyme. That it has not been reported in other echinodermata, or in unrelated types of marine invertebrates, is readily explained by the fact that no assays have been, or seem likely to be, carried out. This type of investigation would serve no practical purpose but might throw light on the present very confusing distribution of thiaminase in nature and on its function in metabolism.

Sautier (1946) included 3 species of starfish from Alaskan waters in his list of fishery products assayed for thiamine by the thiochrome method. These species, _Pisaster giganteus_, _Pisaster ochraceus_, and _Phycnopodia helianthoides_, were reported to contain thiamine within the range of 6 to 17 micrograms per 100 grams (5 assays).

The only other reference to the presence of thiamine in starfish, is that of Hutchinson, _et al_, (1946) who reported 1 milligram per kilogram of starfish meal. They state that, "The low thiamine content of the dried starfish is almost certainly due to post-mortem loss."

Myers (1946), of this laboratory, has found that the thiochrome assay as generally used is of doubtful applicability to the assay of thiamine in fishery products. It is possible that some starfish may contain thiamine and others thiaminase, as similar apparent contradictions have been reported in regard to some species of fish. There are insufficient data to determine the true status of thiaminase, and to correlate its occurrence to such variables as sex, season, maturity, locality, and species. It is suggested that in any fishery product for which a thiamine assay by chemical methods indicates a value of less than 25 micrograms per 100 grams, that the material be tested for the presence of thiaminase by the recovery of added thiamine after incubation under the conditions promulgated for the assay of the enzyme (Sealock, _et al_, 1943).

## THIAMINASE IN STARFISH

The presence of thiaminase in starfish was first detected in the course of a bio-assay intended to determine the thiamine content of raw starfish (Lee, 1948-B). Rats had been maintained on a thiamine test diet (U. S. Pharmacopoeia XI) for a depletion period of 28 days. After this period, rats were assigned to test groups if their weight was less than 100 grams for females, 105 grams for males even though they were still gaining in weight. This unorthodox procedure was necessary in order to assemble a test group within a reasonable time, as the available strain of the rats were quite resistant to thiamine depletion.

Groups of six rats were used on each test level, the animals being evenly distributed as to sex, with no more than one rat from any litter in a group. Rats were housed in individual cages in a room with temperature maintained at 80° ± 2° F. The assay period was four weeks, with liveweight and food consumption records taken weekly.

The first series was composed of ten groups. All rats received the basal thiamine test diet. Three groups received as supplement ½, 1, and 1½ grams of raw starfish per day, 3 groups were given 20, 50, and 80 micrograms of thiamine per 100 grams of diet, and 3 groups received ½, 1, and 1½ grams of raw oysters as a supplement.

The control rats all continued to gain weight during the test period, averaging 8.1 grams per week. The groups receiving thiamine made much larger gains. Groups receiving 20, 50, and 80 micrograms gained 14.1, 20.8, and 24.0 grams per rat per week, respectively. All rats fed oysters also gained more than the controls.

The rats fed raw starfish, however, did very poorly. Of those receiving ½ gram per day, 1 rat gained in weight, 4 rats lost 8 to 27 grams from their starting weight, although they lived for the 4-week period. One rat was killed on the 23rd day after a loss in weight of 27 grams. When one gram of starfish was fed per day, one rat again gained weight, one lost 39 grams before death on the 20th day of test, and 4 others lost 11 to 29 grams during the 4 weeks. One of these latter rats developed severe polyneuritis, but the symptoms were alleviated and there was a slow gain in weight for 2 weeks after it was given 10 micrograms of thiamine in a single dose. When 1½ grams of starfish was fed daily, the final liveweights were 20 to 34 grams below the starting weights and 4 of the 6 rats developed symptoms of acute polyneuritis. The growth curves for these groups are shown in Figure 1.

In view of the evidence that these rats were suffering from an acute thiamine deficiency, 12 of the rats which had been losing weight were selected from the groups fed starfish and were divided into 2 sub-groups. These were fed the basal diet plus 150 micrograms of thiamine per 100 grams of diet with 1 group getting, in addition, 1 gram per day of raw starfish. In 2 weeks, the former sub-group made an average gain of 66.6 grams, while those getting both thiamine and starfish gained 58 grams.

In the meantime, to determine the possible presence of directly toxic agents in the starfish, 2 groups of 11 rats each were fed the regular stock diet, with 1 group getting a daily supplement of 1 gram of raw starfish per rat per day. Over a 3-week period, the controls averaged 23.7 grams per week, and the starfish group, 22.4 grams per week, an insignificant difference.

Considerable difficulty was experienced in getting the rats to eat the starfish supplements, the material apparently was quite unpalatable. This was particularly true of those rats with unlimited access to the stock diet. A number of the animals receiving depletion diets also refused to eat part or all of the starfish offered. This was notably so for those few rats in these groups which gained weight during the test period.

On the basis of these tests, it was evident that there was present in starfish a substance capable of destroying a limited amount of thiamine. These studies had been started in November 1941, at which time "anti-thiamine," as it was then called, had been reported but very little was known regarding its chemical structure or mode of action. Chemical assay methods had not been developed, so that it was necessary to use the biological assay method for thiamine. By the use of two series of test groups supplemented with thiamine, one with and the other without an additional supplement of raw starfish, it should have been possible to determine the thiamine destroyed per gram of starfish.

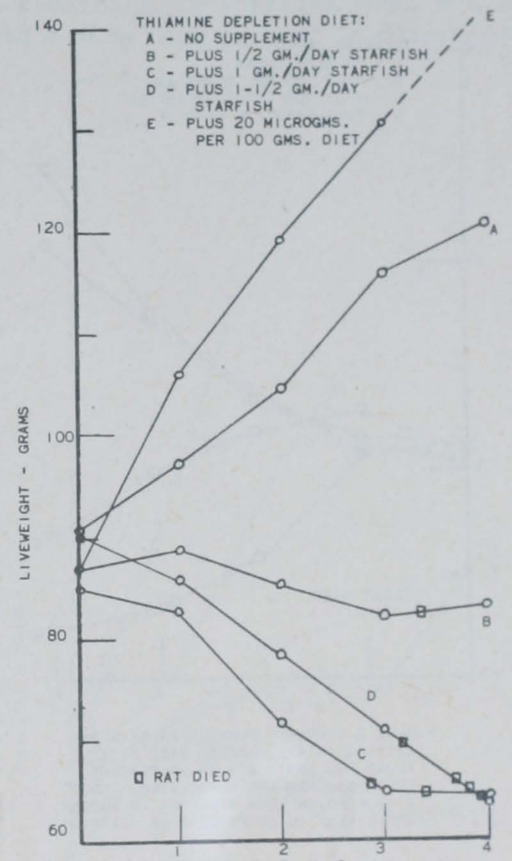


FIGURE 1 - GROWTH CURVES FOR RATS FED THIAMINE BASAL TEST DIET ALONE, AND WITH SEPARATE DAILY SUPPLEMENTS OF RAW GROUND STARFISH AS INDICATED.

This method did not succeed because of the refusal of most of the rats to eat the starfish supplement with sufficient regularity to permit any degree of quantitative comparison. The basal depletion diet was fed alone and with 10, 30, and 60 micrograms of thiamine per 100 grams, with 4 other groups being fed the same diets plus 1 gram per day of raw starfish. During the shortened test period, each of the groups fed starfish gained less weight than the corresponding groups without starfish but the data showed no significant correlations.

A third series of rats was used to determine the practicability of mixing the ground starfish directly into the basal diet, also to ascertain the reported heat lability of thiaminase. Four groups of 6 rats each were used; these were fed the basal thiamine test diet alone; with 10 percent raw starfish; with 10 percent of starfish which had previously been autoclaved for 15 minutes at 15 pounds pressure; and with an amount of starfish oil equal to that which would be introduced by the inclusion of 10 percent starfish in the diet.

Of course, with finely ground starfish mixed into the diet, the only alternative to eating the mixture was fasting. The amount eaten varied depending upon the condition of the rats. It is probable that the starfish produced a depressing effect upon the appetite, in addition to and before the onset of the anorexia resulting from the thiamine deficiency due to the action of the thiaminase.

The control rats in this group gained an average of 5.8 grams per rat per week during the test period; whereas, those fed raw starfish lost 2 to 34 grams, the average loss being 6.7 grams per week. One rat died after 2 weeks on test while another developed polyneuritis in 3 weeks. The group fed the diet containing cooked starfish had an average loss in weight of 1.5 grams per week, two rats making small gains while the other four lost from 3 to 19 grams. When fed the diet containing starfish oil, 5 of 6 rats gained weight, averaging 7.1 grams per week. The growth curves for these groups are shown in Figure 2.

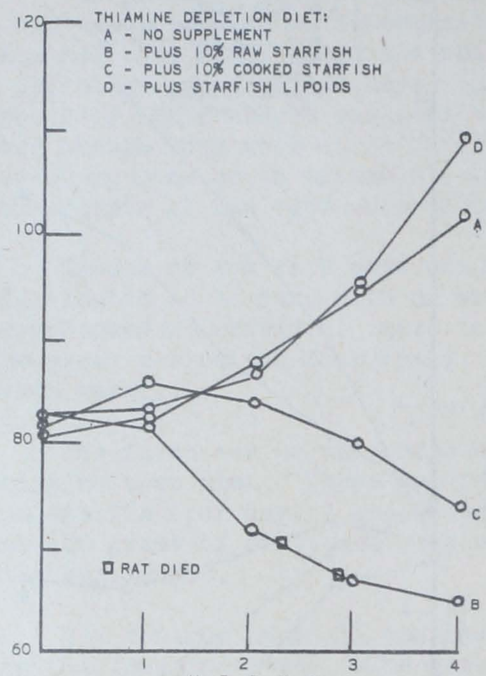



FIGURE 2 - GROWTH CURVES FOR RATS RECEIVING THIAMINE BASAL TEST DIET ALONE AND ADMIXED WITH 10 PERCENT STARFISH, RAW AND AUTOCLAVED, ALSO WITH LIPOIDS FROM STARFISH IN AMOUNT EQUIVALENT TO 10 PERCENT OF STARFISH.

On the basis of this test, it was concluded that starfish oil was in no part responsible for the poor growth which resulted when raw starfish was fed. The destructive principle was not as rapidly and completely destroyed by autoclaving as had been expected, on the basis of reports of heat lability of thiaminase in other materials. This greater heat stability was later confirmed when considerable amounts of thiaminase were found in ground dried starfish meal (Lee, 1948-B).

Relatively heat stable forms of thiaminase have more recently been reported in Indian oil seeds (Bhagvat and Devi, 1944) and in a fern, Pteris aquilina (Weswig, et al, 1946). It now seems probable that "thiaminase" is not a single enzyme, but rather that there are a number of thiamine-destructive principles differing in heat stability, properties on dialysis, and presumably in chemical structure.

Another attempt at a quantitative determination of the thiaminase in starfish was made with a fourth series of rats fed on the thiamine test diet plus 10 percent ground raw starfish, and a group of thiamine supplements. From 10 to 100 micrograms of thiamine per 100 grams were fed in the diet containing starfish, with a control group being fed the basal diet plus 25 micrograms of thiamine per 100 grams. A new lot of starfish had been obtained for this test to lessen the chance of loss of thiaminase which may have occurred in handling or storage of the first lot. The test indicated, however, that the new starfish sample contained less thiaminase than the previous sample. The group fed the basal diet plus 10 percent starfish of the fourth series showed a loss of weight of 2.4 grams per rat per week, only 36 percent of the weight loss of the group in the preceding series fed an identical diet except for the lot of starfish.

The growth curves of this test series are shown in Figure 3. About the best estimate of the amount of thiamine destroyed is 4 micrograms per gram of starfish. The fact that the thiaminase content was lower than was expected led to a selection of supplement levels with too great a spread for accurate evaluation in this low range. The test was not repeated, however, as the procedure was not satisfactory when raw starfish was fed, and results did not justify use of further time and additional rats for this purpose.

## THIAMINASE IN STARFISH MEAL

It has already been noted that thiaminase remaining in starfish meal was a factor adversely affecting the growth of chicks fed a mash formula used to determine the value of the meal as a protein supplement. These chicks had been fed on high levels of starfish meal; namely, 32 percent regular meal and 29.5 percent extracted meal. These levels were fed primarily to determine the tolerance of the chick for excess calcium, and an unbalanced calcium:phosphorus ratio. Thiamine in the mash was supplied by bran, middlings, and other grain products, and was adequate in the diets containing pilchard meal and the lower levels of starfish meal.

There were no deaths in these groups until the 13th day of test, but 11 of 21 chicks died during the next 8 days. It was thought possible that this heavy mortality after 2 weeks might be due to a thiamine deficiency resulting from the presence of thiaminase which had not been destroyed by the low drying temperatures. After 3 weeks, therefore, 100 micrograms of thiamine per 100 grams of mash was added to both diets containing the high levels of starfish. The surviving chicks showed much improvement in condition and fairly good growth response during 5 weeks they were fed on the diets supplemented with thiamine as shown in Figure 4.

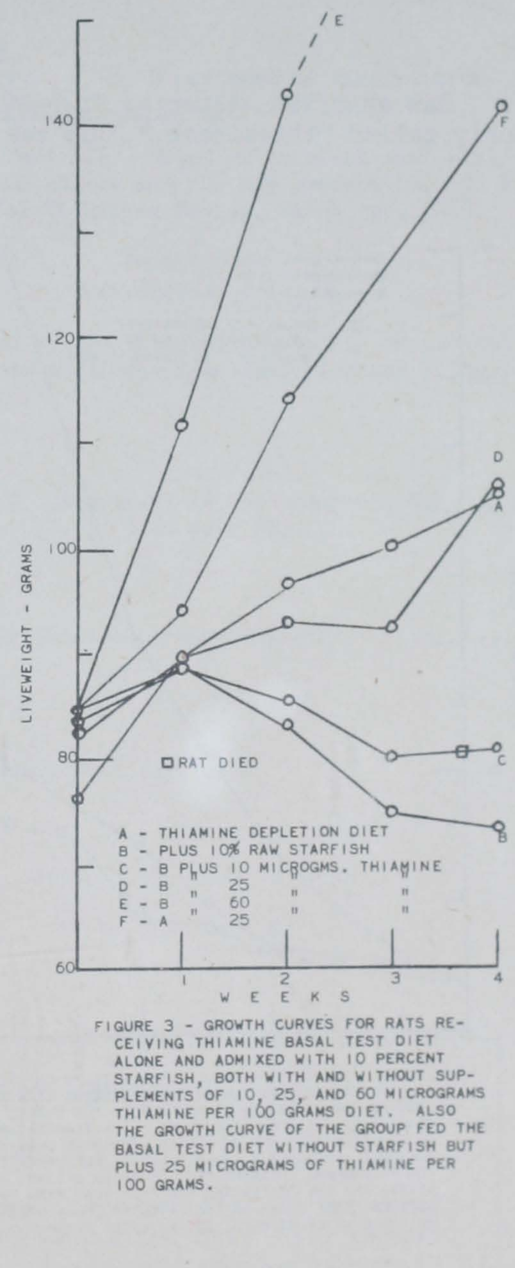



FIGURE 3 - GROWTH CURVES FOR RATS RECEIVING THIAMINE BASAL TEST DIET ALONE AND ADMIXED WITH 10 PERCENT STARFISH, BOTH WITH AND WITHOUT SUPPLEMENTS OF 10, 25, AND 60 MICROGRAMS THIAMINE PER 100 GRAMS DIET. ALSO THE GROWTH CURVE OF THE GROUP FED THE BASAL TEST DIET WITHOUT STARFISH BUT PLUS 25 MICROGRAMS OF THIAMINE PER 100 GRAMS.

The slightly better growth of the group fed the extracted meal was probably due to the extraction or destruction of some thiaminase during the prolonged extraction with hot acetone. The thiamine supplement was therefore doubled for the group fed the regular meal after 2 weeks on the 100-microgram level, and a further response in growth was obtained (see Figure 4).

Growth, even when the diet was supplemented with 200 micrograms of thiamine per 100 grams of mash, did not approach normal for the age of the chicks. There is no proof that even this relatively large amount of thiamine supplied an optimum level. Feeding tests of the same type conducted by other investigators have shown that the addition, for example, of 200 micrograms of thiamine to a mixture containing thiaminase may result in the loss of 90 percent, which is equal to 180 micrograms of thiamine. If much larger amounts, perhaps 1,000 micrograms, were added, larger actual quantities, but a smaller proportion of the total, for example, 700 micrograms, may be destroyed by the same amount of thiaminase.

On the other hand, the excess calcium carbonate, amounting to almost 18 percent of the diet, was undoubtedly also a factor tending to retard growth but the separate evaluation of the two effects is impossible from the present data. It is evident, however, that the mortality was primarily the result of a thiamine deficiency due to the thiaminase content of the starfish meal rather than to excess calcium.

## CONCLUSIONS

Raw starfish, Asterias forbesi, contains a thiamine-destructive enzyme, commonly called "thiaminase." This was demonstrated by the development of polyneuritis in rats fed one or more grams of raw starfish per day. Recovery and normal growth resulted from the addition of an adequate level of thiamine to the diet.

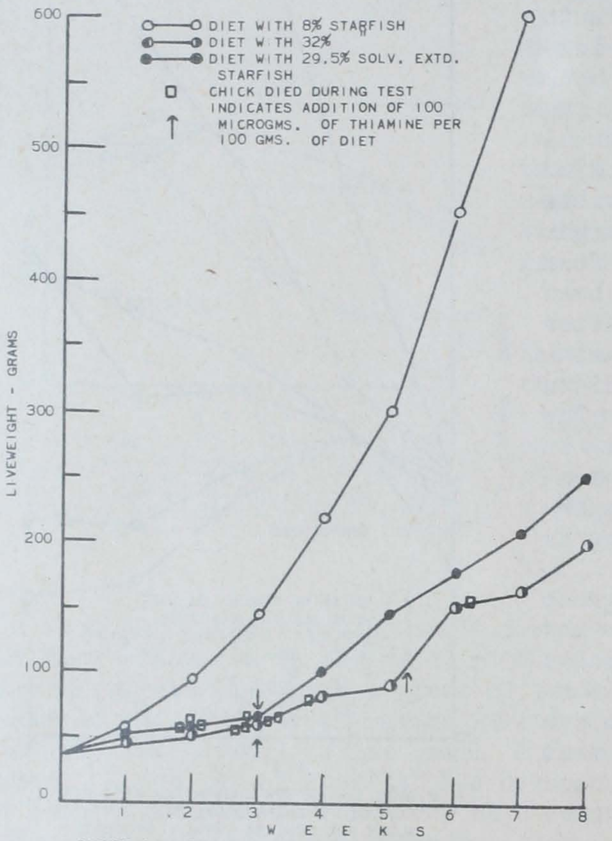



FIGURE 4 - GROWTH CURVES FOR NEWLY HATCHED CHICKS, FED EQUI-PROTEIN MASHES CONTAINING 8 PERCENT STARFISH MEAL, SHOWING NORMAL GROWTH. ALSO CURVES FOR CHICKS FED 32 PERCENT STARFISH MEAL DRIED AT A LOW TEMPERATURE, AND 29.5 PERCENT OF SAME MEAL WITH LIPOIDS EXTRACTED BY ACETONE. THE LATTER TWO DIETS WERE SUPPLEMENTED BY 100 MICROGRAMS THIAMINE PER 100 GRAMS OF DIET AFTER 3 WEEKS, AND THE 32 PERCENT STARFISH MEAL MASH HAD THE SUPPLEMENT OF THIAMINE INCREASED TO 200 MICROGRAMS OF THIAMINE AFTER 37 DAYS, AS INDICATED BY ARROWS.

It was difficult to obtain a quantitative estimate of the amount of thiamine destroyed because of the refusal of rats to eat the raw starfish with any regularity. The best estimate is that one gram of starfish inactivates four micrograms of thiamine. There are indications that other samples of starfish may contain greater amounts of thiaminase.

The thiaminase in starfish is sufficiently stable to remain in starfish meal that has been sun-dried or dried at low temperatures. There are no data to show the amount of thiaminase lost under different conditions of drying.

Thiaminase in starfish meal is primarily responsible for mortality of chicks fed diets containing high levels of this product. This factor and the high calcium content of the starfish meal are responsible for the poor growth obtained. Evidence from other sources indicates that thiaminase would be destroyed at the temperatures commonly used in the dryers in the commercial production of protein meals from fishery products. Any small amount of thiaminase remaining would not give much response since usually less than 10 percent of mixed animal protein supplements are included in commercial mash formulas.

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## CHEMICAL COMPOSITION OF SOME CANNED FISHERY PRODUCTS

The dry matter content of the different types of fish varied considerably, from the minced razor clams which contained only 13.9 percent, to the oil packed bluefin tuna which was almost half dry matter or 47.2 percent. Generally speaking, the fatty fish--salmon, herring, mackerel, sardines, and menhaden--are high in dry matter and in energy value. This is true because the protein content of all species is fairly constant, at 17 to 20 percent, higher only when fish have had a precook and partial dehydration. The fat content, which may range up to 20 percent, is in addition to rather than a replacement of any part of the protein.

--Fishery Leaflet 295