AMINO ACID COMPOSITION OF THE PROTEIN AND INORGANIC CONSTITUENTS OF THE ASH OF POLLOCK FISH SCALES

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ABSTRACT

THE AMINO ACID COMPOSITION OF THE PROTEIN OF POLLOCK FISH SCALES WAS DETERMINED BY PAPER PARTITION CHROMATOGRAPHY AND MICROBIOLOGICAL ANALYSIS. THE DATA INDICATE THAT THE PROTEIN OF POLLOCK FISH SCALES IS PROBABLY A SCLEROPROTEIN OF THE COLLAGEN TYPE CONTAINING HIGH LEVELS OF GLYCINE AND AMOUNTS OF ARGININE AND SERINE ABOUT EQUAL TO THAT FOUND IN THE PROTEIN OF WHOLE EGG. THE SCALE PROTEIN CONTAINS FAIR AMOUNTS OF ALANINE, PROLINE, AND HYDROXYPROLINE, AND EXTREMELY SMALL AMOUNTS OF CYSTINE AND TRYPTOPHAN. ALSO, THE SCALE PROTEIN CONTAINS SLIGHTLY LESS ASPARTIC ACID, GLUTAMIC ACID, AND THREONINE, AND MUCH SMALLER AMOUNTS OF HISTIDINE, ISOLEUCINE, LEUCINE, LYSINE, METHIONINE, PHENYLALANINE, TYROSINE, AND VALINE THAN DOES WHOLE-EGG PROTEIN. THE INORGANIC CONSTITUENTS OF THE ASH OF POLLOCK SCALES WERE DETERMINED SEMIQUANTITATIVELY BY SPECTROGRAPHIC ANALYSIS. THE DATA INDICATE THAT NO UNUSUAL KINDS OR QUANTITIES OF INORGANIC CONSTITUENTS THAT MIGHT CAUSE BIOLOGICAL INJURY WHEN FED TO ANIMALS ARE PRESENT IN THE ASH OF THE SCALES. AN INTERPRETATION OF THESE FINDINGS IS MADE IN RELATION TO CONCLUSIONS OBTAINED FROM A PREVIOUSLY REPORTED RAT-FEEDING STUDY ON THE COMPARATIVE NUTRITIVE VALUE, DIGESTIBILITY, AND "BIOLOGICAL VALUE" OF POLLOCK-FISH-SCALE AND CASEIN-LACTALBUMIN PROTEINS.

INTRODUCTION

During the past few years, the fishing industry has had the increasingly difficult problem of annually disposing of thousands of tons of fish scales because filleted fish now have replaced unprocessed fresh fish in sales volume. Harbors that are close to plants normally provide an inexpensive area of scale disposal, but unless tidal flows are strong, pollution may result.

FIG. 1 - PAPER PARTITION CHROMOTOGRAPHY IS USED TO IDENTIFY UNKNOWN AMINO ACIDS IN PROTEIN HYDROLYSATES.

Chemical engineers and pharmaceutical houses have been contacted to obtain suggestions for utilizing the scales, but this approach has been unsuccessful. Attempts to use the scales as fertilizer on local farms also have not met with success.

In the past, very little research has been conducted with fish scales. The calcium oxalate and total calcium contents were determined in the scales of 30 Japanese species of teleosts (Nishihara 1954). Obata et al (1950 and 1953) and Groen (1953) studied the identity of the pearl essence of fish scales. Corti and Keller (1952) chromatographed fish-scale hydrolysates and identified a few of the amino acids present. A purple or blue fluorescent substance in the skin and scales of some fish was studied by several investigators (Fontaine and Busnel 1938, Polonovski et al 1943, and Hama et al 1952). Block et al (1949) showed that herring scales contain gelatin and a collagen-like protein, namely ichthylepiden. Randoin et al (1938) reported that the skin and scales of certain fish contain a flavin that can be utilized by rats in place of riboflavin. Nichols (1956) investigated to a limited extent the physical structure of fish scales. Thus, the information found in the literature is brief and is of little value in suggesting a solution to the present problem.

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Recently, however, as part of a general study to determine whether scales may have value as a supplemental source of protein in the diets of animals, Snyder and Nilson (1957) conducted rat-feeding tests to compare the nutritive value for growth, the biological value for maintenance, and the digestibility of pollock-fish scale and casein-lactalbumin proteins. The data from these studies indicate that pollock-fish scale protein is digested about 80 percent, but is utilized about 30 percent less efficiently than is a protein supplement consisting of three parts casein and one part lactalbumin (CL). The data also indicate that fish scales as the only source of protein in a diet containing 9 percent protein are incapable of supporting growth of young rats but can be utilized as a limited source of protein when supplemented with CL protein. Increased utilization of pollock-fish scale protein, in combination with stepwise higher levels of CL protein, indicate that no toxic substances per se for growing rats are present in the scales.

The nutritional inadequacy of pollock-fish scale protein alone and the increased utilization of that protein in combination with stepwise higher levels of CL protein were interpreted as likely due to a deficiency and/or imbalance of specific nitrogen nutrients in pollock-fish scales. This interpretation was supported also by information obtained in the literature concerning deficiency states caused by feeding similar types of waste protein that serve a protective function in the animal organism, such as wool (Routh 1942), hog hoofs (Wagner and Elvehjem 1942, 1943), and chicken feathers (Wilder et al 1955). Chemical analyses of similar proteins that have a supporting function in the animal organism, such as collagen (Bowes et al 1955) and elastin (Graham et al 1949) have indicated deficiencies of some "essential" amino acids.

Information on the amino acid-composition of pollock-fish scale protein was considered necessary, therefore, to evaluate properly the conclusions suggested from the results of the rat-feeding study. A knowledge of the inorganic constituents present in the ash also was deemed desirable, since the scales contain nearly 40-percent ash, presumably as various apatites (Corti and Keller 1952), which might contain unusual inorganic constituents or great excesses of other constituents that could affect nutritive quality.

MATERIAL AND ANALYSIS

The fish scales received at this laboratory for these studies were furnished by the staff of the Fishery Technological Laboratory, U. S. Bureau of Commercial Fisheries, East Boston, Mass. Pollock (Pollachius virens) were scaled by hand. The scales were washed thoroughly with water, drained, and spread in pans to dry in an oven at 100° C. They then were shipped to this laboratory, where they were ground, as finally as possible, in a Hobart coffee grinder.

No great variation was found in the moisture, protein (N x 6.25), fat, and ash of representative samples of scales from the various lots. The mean and ranges of moisture content for 4 lots of scales were 4.4 and 1.0-6.9 percent, respectively; the protein content for 11 lots was 59.5 and 56.6-62.5 percent; the fat content for 4 lots was 0.004 and 0.0-1.0 percent; and the ash content for 5 lots was 38.9 and 36.1-43.1 percent. The methods of analyses of the Association of Official Agricultural Chemists (1955) were used.

A representative sample of ground scales was chosen randomly from one of the lots received at this Laboratory for use in the present study. The means of three analyses each of this sample of scales on a moisture-free basis were 60.1 percent for protein and 39.4 percent for ash.

No significant difference was found between the nutritive value of the scale protein from the lot of scales from which this sample was obtained and that of the various other lots of scales. It can be concluded, therefore, that the sample from this lot of scales was representative of the scales of pollock in general. The small dif-

ferences in moisture, protein, fat, and ash contents found among the various lots of scales probably can be attributed to the nutritional status and age of the fish from which the scales were collected (Nishihara 1954).

EXPERIMENTAL AND RESULTS

PAPER-PARTITION CHROMATOGRAPHY: Initially, paper-partition chromatography was employed to determine the amino acids present in an acid and alkaline hydrolysate of pollock fish scales. Whatman No. I paper for chromatography was used with the following solvent systems, either employed separately for one-dimensional, or in various combinations for two-dimensional chromatography: (1) phenol; water (80/20 v/v) to which was added 0.004 percent 8-hydroxyquinoline; (2) as above, but with a beaker of 0.3 percent NH_4OH introduced into the chromatographic chamber; (3) 1-butanol:acetic acid:water (40:10:50 v/v/v); and (4) 2, 6-lutidine:2, 4, 6-collidine:water: diethylamine (100:100:100:3 v/v/v). The chromatograms were developed by dipping in a 0.25-percent acetone solution of ninhydrin.

Semiquantitative data were obtained by visually comparing a series of dilutions of known and unknown concentrations of the amino acids under investigation on the same one or two dimensional chromatograms (Block et al 1955).

Some difficulty was experienced in interpreting the chromatograms owing to the extremely high content of salt in the scales; but in general, glycine was present in the hydrolysate in great excess, whereas most of the other amino acids were present in lesser amounts than those amounts usually found in natural proteins. Proline and hydroxyproline were present in fair amounts. Cystine and tryptophan either were missing or were present in extremely small amounts.

MICROBIOLOGICAL ANALYSIS: Although the data on amino acid content obtained from chromatographic analyses were enlightening, it was believed that more precise quantitative data obtained from microbiological analyses would better supply the information needed on how to conduct further feeding studies to determine the nature of possible amino acid imbalances in the protein from fish scales. These data also would aid in determining the nature and amount of amino acid supplementation needed to enhance the nutritive value of the pollock-fish scale protein and to reduce imbalances. A sample of the ground pollock fish scales was sent to the Wisconsin Alumni Research Foundation, Madison, Wis., to be assayed for 18 amino acids by a microbiological method. The method of analysis used was that of Henderson and Snell (1948). A single media was employed that contained all the nutrients required for growth by the various lactic acid-producing test organisms used to conduct the assay.

The microorganisms employed were Lactobacillus arabinosus 17-5 for glutamic acid, leucine, phenylalanine, valine, and tryptophan; Streptococcus faecalis R for arginine, histidine, methionine, and threonine; Latobacillus citrovorum for alanine; Lactobacillus casei for serine and Leuconostic mesenteroides P-60 for aspartic acid, lysine, proline, isoleucine, glycine, cystine, and tyrosine.

Table 1 - Amino Acids Per 16 Grams of Nitrogen Present in Pollock-Fish Scale and Whole-Egg Protein[1/]

Amino Acid	Pollock Scales	Whole Egg
	. . . (Grams) . .	
Alanine	4.1	2/
Arginine	8.8	7.0
Aspartic acid . . .	4.6	5.7
Cystine	0.5	2.3
Glutamic acid . . .	10.6	12.6
Glycine	37.6	3.7
Histidine	1.6	2.4
Isoleucine	2.5	7.7
Leucine	3.5	9.2
Lysine	4.4	7.5
Methionine	1.8	4.0
Phenylalanine . . .	2.7	6.3
Proline	3.5	2/
Serine	9.9	7.5
Threonine	4.1	5.0
Tryptophan	0.5	1.7
Tyrosine	2.3	4.5
Valine	3.4	7.8

1/ APPROXIMATELY EQUAL TO PERCENT OF PROTEIN.
2/ NO ANALYSES FOUND.

The results of the microbiological assay, as grams of amino acid per 16 grams of nitrogen, and the amino acid composition of whole-egg protein (Hawk et al 1954) are included in table 1. Whole-egg protein is considered by many to be an ideal protein and thus should be an excellent protein for comparison and reference.

The amino acid analyses show that the pollock-fish scale protein is rich in glycine and contains about the same amount of arginine and serine as does whole-egg protein. Pollock-fish scale protein contains slightly less aspartic acid, glutamic acid, and threonine, and much smaller amounts of isoleucine, lysine, methionine, phenylalanine, tyrosine, and valine. Extremely small amounts of cystine and tryptophan are present.

SPECTROGRAPHIC ANALYSIS: The inorganic constituents of the two samples of ashed scales from the same lots were determined semiquantitatively by spectrographic analysis in a Bausch and Lomb quartz spectograph with direct arc excitation. The data were kindly furnished by Maurice J. Peterson of the U. S. Bureau of Mines, College Park, Md. The first sample of scales was dry-ashed at 550° C., and the second sample was wet-ashed with sulfuric and nitric acid at about 200° C.

Each of these samples of ashed scales was mixed with graphite powder of high purity and the mixtures were placed in a graphite electrode. The mixture in the graphite electrode then was arced against a graphite counter-electrode, using direct-current arc excitation, with the current being approximately 12 amperes. The completed vaporization of the sample required about 2.5 minutes. While the arcing was taking place, the spectrograph recorded on photographic plates the spectrum of the elements present in the sample.

Two different types of Eastman plates were used to record the spectrum from 2400 to 8600 Ångstrom units: A spectrum analysis number 2 plate was used for the ultra-violet and blue region, and an I-L plate was used for the remainder of the blue region to 8600 Ångstrom units.

These photographic records, or spectrograms, were compared visually with spectrograms of varing concentration standards of mineral elements, the wavelengths of which were recorded under identical conditions on separate plates. The semiquantitative results of the spectrographic analyses are presented in table 2.

Table 2 - The Percentage Range of Inorganic Constituents Present in the Ash of Pollock Fish Scales

	Ca	P	Mg	Mn	Fe	Al	Cu	Ni	Na	Ba	Sr	Li	K	Si
Dry ashed	>10	>10	0.3-3.0	.01-.10	.03-.30	.01-.1	.005-.05	-1/ -	.03-.30	.001-.01	.3-3.0	.005-.05	.01-.10	.01-.10
Wet ashed	>10	>10	.3-3.0	.01-.10	.03-.30	.02-.2	.005-.05	.005-.05	.03-.30	.001-.01	.3-3.0	.005-.05	.1-1.0	.05-.50

1/ NOT DETECTED.

Manganese, copper, barium, and lithium were present in amounts of less than 0.1 percent by weight in both samples of ashed scales. Nickel was present in a similar amount in wet-ashed scales, but was absent in the dry-ashed scales. Aluminum, potassium, and silicon were present in amounts of less than 0.1 percent in the dry-ashed, but were present in slightly greater percentages in the wet-ashed scales. Strontium and magnesium were present in amounts of less than 3.0 percent in both samples. Calcium and phosphorus were present in amounts of more than 10 percent in both samples.

CONCLUSIONS

Disposal problems associated with an accumulation of waste fish scales at filleting plants have stimulated an investigation of the value of scales as a supplemental source of protein in the diets of farm animals.

As an initial study in this investigation, Snyder and Nilson (1957) conducted rat-feeding tests to compare the nutritive value for growth, the biological value for maintenance, and the digestibility of pollock-fish scale protein and a protein supplement consisting of three parts casein and one part lactalbumin (CL). They found the mean apparent digestibility of pollock-fish scale protein to be nearly 80 percent when fed to male and female rats at a 9.00-percent level in the diet. Pollock-fish scale protein was found to be completely digested, as indicated by true digestibility values, when only enough was fed to equal metabolic nitrogen. The initial amino acid analyses by paper-partition chromatography reported herein apparently supplies a reasonable explanation for this rather unexpectedly high level of digestibility of scale protein. The presence in extremely small quantities of tryptophan and cystine and in large quantities of glycine and the presence of fair amounts of hydroxyproline suggest that pollock-fish scale protein is not a keratin that would be digested with difficulty, but probably is a scleroprotein of the collagen type that would be digested easily.

This classification of fish-scale protein is not necessarily original, since Block et al in 1949, from chemical analyses alone, reported that herring scales contained a collagen-like protein. The classification of scale protein as keratin has persisted, however, probably owing to anatomical and presumed embryological considerations. Also, the studies of Block and his co-workers may not have received sufficient publicity, or perhaps, supplied enough evidence to warrant classification of fish-scale proteins as collagens. The results of the amino acid analysis presented herein and the digestibility studies presented earlier indicate rather strongly, however, that fish-scale protein is most likely a scleroprotein of the collagen type and not a keratin. These conclusions are based on interpretations of results from pollock scales only, but probably apply to scales from all species of fish.

The quantitative amino acid content found probably also explains why, in the rat-feeding study reported earlier, pollock-fish scale protein alone was not adequate for growth and why the utilization of that protein was increased when combined with stepwise higher levels of CL protein. The microbiological as well as the paper chromatographic analyses show that the pollock-fish scale protein is deficient in all the so-called essential amino acids, except arginine, and in some of the nonessential amino acids when compared to whole-egg protein. These deficiencies in essential amino acids also would explain the decreased assimilation by rats of pollock-fish scale protein as compared to CL protein.

The fact that scale protein contains nearly 40-percent glycine suggests a possible glycine imbalance or toxicity when the scales are fed at high levels. This, in turn, may cause the manifestations of other nutritional inadequacies of pollock-fish scale protein.

The results of the spectrographic analyses suggest that no unusual inorganic constituents, or great excesses, are present in pollock fish scales that would cause biological injury when fed to animals. This conclusion is also in accord with the conclusions based on results from the rat-feeding study; that is, that scales contain no toxic substances per se for growing rats.

The evidence presented herein therefore supports the conclusions arrived at from interpretations of the results of the rat-feeding study. The fact that the pollock-fish scale protein is well digested but is not utilized by rats when fed alone, and yet is increasingly better utilized when fed in combination with higher levels of CL protein, apparently is explained by classifying pollock-fish scale protein as a collagen that is deficient in many essential amino acids and imbalanced in glycine content. The results of the spectrographic analyses, in turn, do not indicate that the concentrations of the various inorganic elements are likely to cause the depression of growth that was noted when the diets containing the pollock fish scales were fed to the rats.

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NORTH ATLANTIC SEA SCALLOP FISHERY

Most of the scallop-fishing vessels out of New Bedford, Mass., use two dredges, each 11 feet wide. They are towed together, one from each forward gallows frame, and brought up alternately to be dumped on deck. The dredges are very sturdy so that they will stand up under the rough use that they get. A complete 11-foot dredge with 3-inch rings weighs about 1,400 pounds when empty. Some of the smaller boats with less power use 8- or 10-foot dredges and 13-foot models have been tried on the larger boats but the 11-foot dredge is now fairly standard in the New Bedford fleet. There is really no standard design for a dredge; each fishing captain has his own ideas on what makes for efficient gear and modifies and alters the basic design to suit himself.

--Fishery Leaflet 442, Sea Scallop Boats and Gear, August 1957.