RAT BIOASSAY OF UNIDENTIFIED GROWTH FACTORS IN POLLOCK FISH SCALES

By Donald G. Snyder*

ABSTRACT

Data are presented that indicate the presence of fair amounts of unknown growth factors in pollock fish scales when fed to rats in a special diet containing a thyroid stress factor. Thus, in addition to earlier feedings, further evidence is presented to suggest that fish scales may have value as a feed supplement in the diets of farm animals.

The problem of disposing of waste fish scales at filleting plants has stimulated an investigation of the value of the 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 (1) the nutritive value for growth, (2) the "biological value" for maintenance, and (3) the digestibility of pollock-fish-scale (PFS) protein and a protein supplement consisting of 3 parts casein and 1 part lactalbumin (CL).

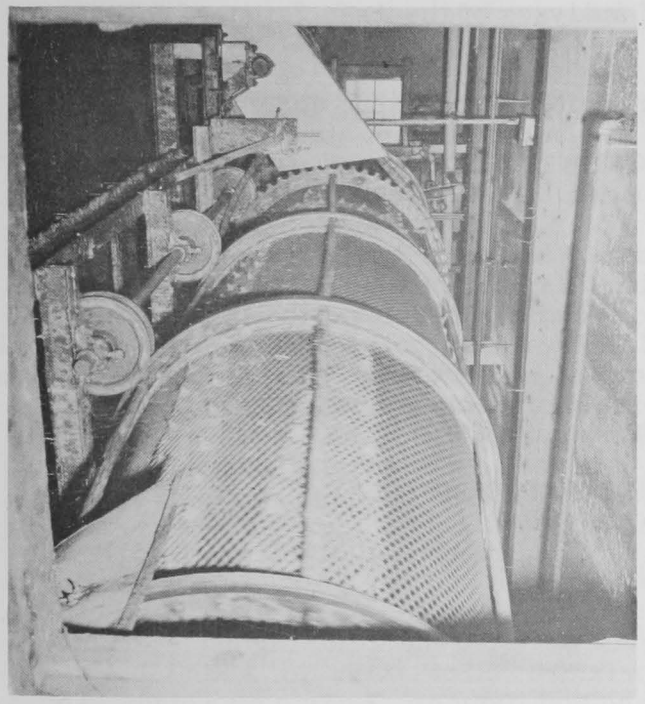

Fig. 1 - During commercial operations, fish scales generally are collected from expanded metal cylindrical automatic scalers.

The data from these studies indicate that PFS protein is digested as well as is the protein from CL but is utilized about 30 percent less efficiently. The data also indicate that young rats do not live when fed a diet containing 9 percent protein from only the protein of PFS. The protein from PFS can be utilized by the rats, however, when supplemented with CL protein in the diet. Increased utilization of PFS protein, in combination with stepwise higher levels of CL protein, indicates that no toxic substance per se for growing rats are present in the scales.

The nutritional inadequacy of PFS protein alone and the increased utilization of PFS protein in combination with stepwise higher levels of CL protein were interpreted as likely to be due to a deficiency and/or imbalance of specific nitrogen nutrients in PFS. Data in support of this interpretation were obtained in a further study on the amino acid composition of the protein of PFS (Snyder 1958). These data indicate that the protein of PFS is probably a scleroprotein of the collagen type, which contains high levels of glycine and low levels of many of the other amino acids. The data also indicate that the scales contain no unusual kinds or quantities of inorganic constituents that might cause biological injury when fed to animals.

The present study provides data that indicate the presence of fair amounts of unknown growth factor(s) for rats in the pollock fish scales. A qualitative estimate is given, since it has not been possible with the present bioassay methods to give a quantitative value.

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MATERIAL

The pollock fish scales were furnished by the staff of the Bureau's Fishery Technological Laboratory, 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. The scales then were shipped to this laboratory, where they were ground, as finely 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 four lots of scales were 4.4 and 1.0-6.9 percent respectively; of protein content for 11 lots were 59.5 and 56.6-62.5 percent; of fat content for four lots were 0.004 and 0.0-1.0 percent; and of ash content for five lots were 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 the 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 differences 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

The method used in this study was that of Emerson and Folkers (1951), with modifications. Post-weaning, black-hooded male and female rats were allotted to the bioassay at initial weights of 51 to 53 grams and 48 to 51 grams, respectively. During a 28-day depletion period, the rats were fed the following depletion diet in parts per 100: soybean meal, 60; salt mixture, U.S.P. XIV, No. 2 for vitamin A bioassay, 4; dextrose, 24; Crisco, 10; and cod liver oil, 2 parts by weight. To every 100 grams of this basal diet was added 0.075 grams of the following vitamin mixture: thiamine HCl, 1; riboflavin, 2; pyridoxine, 1; calcium pantathenate, 10; nicotinamide, 10; inositol, 5; para-aminobenzoic acid, 30; biotin, 0.05; folic acid, 0.2; menadione, 14.2; ascorbic acid, 2; and vitamin B_{12}, 0.01 parts by weight. In addition, 0.35 grams of choline chloride, 0.015 grams of alpha tocopherol, and 0.25 grams of thyroid powder were added. The thyroid powder was added as a stress factor to increase the demand of the rat for growth factors and, thereby, permit more sensitive detection.

At the end of the depletion period, the male and the female rats were separated as to sex, randomly allotted into groups of three rats each and fed the following four test diets (table 1) during a repletion period of 14 days: (1) depletion diet; (2) CP-50, 10 percent; (3) PFS, 5 percent; and (4) PFS, 10 percent. The "depletion diet" was the one given above. It contained no test material. The "CP-50, 10 percent" included 10 percent by weight--at the expense of soybean meal--of a sample of commercial menhaden meal of known origin that has been used at the College Park Laboratory for several years as a reference sample for the bioassay of unknown growth factors present in fish meal. Meal CP-50 contains a fair amount of unknown growth factors. The diet "PFS, 5 percent" and the diet "PFS, 10 percent" included 5 and 10 percent by weight of pollock fish scales at the expense of soybean meal.

The rats were housed individually in wire screen cages fitted on wire mesh floors. The temperature of the room was maintained at 80° F. The rats were supplied with food and water ad libitum, and weekly records were taken of live weight and consumption of food.

The mean actual gains in weight were 10.7, 21.3, 19.0 and 31.0 grams (table 1) for the male group fed the following four respective diets: (1) depletion diet; (2)

Table 1 - Actual and Estimated Mean Gains in Weight for Rats in Bioassay of Unknown Growth Factors in Pollock Fish Scales

Diet Designation	Actual Mean Gain		Estimated Mean Gain		Difference Between Actual and Estimated Mean Gain	
	Male	Female	Male	Female	Male	Female
	(Grams)					
Depletion diet	10.7	7.0	14.6	8.5	-3.9	-1.5
CP-50, 10 percent	21.3	18.7	18.6	18.8	2.7	-0.1
PFS, 5 percent	19.0	16.0	22.1	22.1	-3.1	-6.1
PFS, 10 percent	31.0	23.7	28.4	19.7	2.6	4.0

Note: CP-50 is a menhaden meal containing fair amounts of unknown growth factors. PFS is an abbreviation for pollock fish scales. Values in percent refer to supplementations by weight of the test material in place of a like amount of soybean meal contained in the depletion diet.

CP-50, 10 percent; (3) PFS, 5 percent; and (4) PFS, 10 percent. For the female groups fed the same diets, the mean actual gains in weight were 7.0, 18.7, 16.0, and 23.7 grams, respectively. In general, the increased gains in actual weight of the male and the female groups fed diets containing meal CP-50 and PFS compared to the male and female groups fed the depletion diet indicate the presence of fair amounts of unknown growth factors in PFS. This interpretation appears valid, since meal CP-50 is considered to contain fair amounts of unknown growth factors. Interpretations to determine amounts of unknown growth factors present in meal CP-50 and PFS obtained by comparing difference in actual mean gains of the various groups fed the different diets, however, are limited, owing to the varying amounts of diet consumed by rats in different groups and to the varying initial weights of the rats at the start of the repletion period of feeding.

To eliminate variability of food consumption and weight, the estimated gains were computed from a multiple regression analysis that included initial weights of the rats in each group at the start of the repletion period, food consumed, and gains in weight during the repletion period (Snedecor 1956). Probably the most valid interpretation of the data to determine amounts of unknown growth factors present in PFS, then, is to compare the actual mean gains with these estimated mean gains thus computed of each of the male and the female groups fed the same level of supplementation (table 1). The difference between actual and estimated mean gain was -3.9, 2.7, -3.1, and 2.6 when the male groups were fed the depletion diet; CP-50, 10 percent; PFS, 5 percent; and PFS, 10 percent, respectively. The difference between actual and estimated mean gain was -1.5, -0.1, -6.1, and 4.0 when the female groups were fed these same diets, respectively. These data indicate that for rats, and under these conditions of testing, a diet containing 10 percent PFS contains fair amounts of unknown growth factors but that one containing 5 percent PFS contains insufficient amounts. No reasons are obvious for the large difference (-6.1) between the actual and estimated mean gain of the female groups fed the diet "PFS, 5 percent." These data would suggest that although PFS contains unknown growth factors, sufficient quantities must be included in the diet to insure the effect of these factors on growth.

CONCLUSIONS

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 whole fish in volume of sales. Harbors that are close to plants normally provide an inexpensive area for the disposal of scales, but unless tidal flows are strong, pollution may result. Various attempts to utilize the scales have been unsuccessful.

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 farm animals, Snyder and Nilson (1957) found that pollock-fish-scale (PFS) protein is well digested by rats and can be utilized as a limited source of protein when supplemented with casein-lactalbumin protein. In this paper are presented data that indicate the presence of fair amounts of unknown growth factors in PFS.

It must be pointed out, however, that unknown growth factors in PFS are indicated here only under the conditions of this particular experiment: that is, when rats are fed a special diet containing a thyroid stress factor.

The problem remains to determine (1) the effect of unknown growth factors when diets containing no stress factors are fed and (2) the ability of other species of animals to utilize these growth factors.

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PROCESS TRANSFORMS FISH-LIVER OIL INTO STABILIZED DRY VITAMIN A

A unique process which transforms fish-liver oil into a stabilized dry Vitamin A has been developed by a Tokyo, Japan, firm. The process involves coating minute particles of the oil with selected rigid materials so that the Vitamin A content is not destroyed by contact with the air or with other materials. The stabilized potency of the vitamin and economical prices are expected to result in great advantages to consumers, as the process is utilized in pharmaceuticals and enriching food (Western Fisheries, April 1958).

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