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# SOME STUDIES ON THE FEEDING VALUE OF FISH MEALS

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ABSTRACT

Commercial fish meal prepared from pilchard, menhaden and lean marine groundfish fillet scrap was found to be an excellent source of protein in a properly balanced diet for growing chicks. These fish meals could be fed in fairly large amounts in diets and in only a few instances were fishy or off flavors detected in the flesh of the chicks at the end of six weeks.

Experimental spoilage of these fish meals under conditions of high humidity and heat in an aerobic environment did not produce any toxic substances. Water-soluble growth promoting substances and vitamin K were synthesized by the bacteria and molds during the experimental spoilage of the meal.

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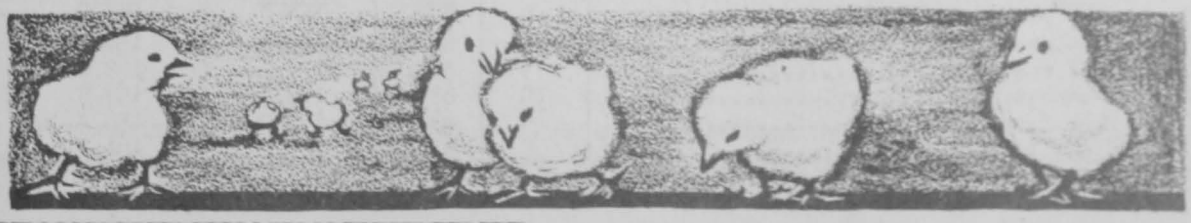
## INTRODUCTION

Lanham and Nilson (1942) reported that chicks fed a semi-purified diet containing experimentally spoiled pilchard meal grew to a larger size, and with lower mortality than those fed a similar diet containing commercial meal. The diets fed contained about the maximum quantity of meal that could be incorporated into an otherwise complete diet; namely, 30 to 35 percent, in order to aggravate any adverse effects of spoilage. The data show that spoilage under aerobic conditions of excess heat and moisture did not adversely affect the nutritive value of the protein of the pilchard meal. It was concluded that any unsatisfactory results obtained in the feeding of diets containing commonly used levels of commercial fish meal are probably due to improper balance of the nutritive elements in the diet rather than to any toxic effect of the fish meal.

The hereinafter reported data are from two types of experiments. First, those from preliminary feeding tests with rats and chicks using meals prepared from pilchard (Sardina caerulea), menhaden (Brevoortia tyrannus), lean marine groundfish fillet scrap (commercial "whitefish" meal), and horseshoe crab (Limulus) in order to determine if any toxic factors may have been produced during experimental spoilage. Secondly, data are included from a rather extensive study with chicks to



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identify the factors which may have been responsible for the better growth of chicks fed the experimentally spoiled meals. The latter study was limited to pilchard meal.

The objects of the investigation were, first, to determine the identity of toxicosis or deficiency state produced in chicks when "commercial" meals were fed in conjunction with otherwise semi-purified foods, and secondly, to determine the identity of the growth factors, probably vitamins, produced during experimental spoilage of meals. During the latter stages of experimental work, the possibility was also explored of using the experimentally spoiled meal as a raw material for extraction of certain vitamins commercially. This phase of the study, however, was dropped when it became apparent that a scarcity of fish meal for feeding purposes would soon develop.

The experiments were conducted between January 1938 and September 1941. The preparation of these data for publication was delayed by the assignment of the authors to other duties during the war. Some of the basic data included in the publication by Lanham and Nilson (1942) are also included in this compilation.

## EXPERIMENTAL METHODS AND DATA

### Rat Feeding Experiment

The rats were allotted at an initial live weight of 48 to 55 g. into six groups of about nine rats each. They were housed individually in cages with screen floors; and, twice weekly records were kept of body weight and food consumption.

The following diet was fed during the 60-day experimental period: fish meal to equal 20 parts protein by weight; sucrose, 20; lard, 12; cod liver oil, 2; salt mixture, U.S.P. XI No. 2, 4; dried brewer's yeast, 2; wheat germ, 1; and dextrin to make 100 parts by weight. This diet was supplemented with 0.12 mg. of thiamine hydrochloride, and 1.2 mg. of crystalline riboflavin per kg. of diet.

Table 1 - Data on Crude Protein (N x 6.25) Content of Meals

| Fish Meal | Percent by Weight | Fish Meal | Percent by Weight |
|---|---|---|---|
| Commercial pilchard meal ..... | 70.1 | Commercial "whitefish" meal[1] .... | 67.6 |
| Spoiled pilchard meal ..... | 69.6 | Spoiled "whitefish" meal[1] ..... | 65.1 |
| Commercial menhaden meal ..... | 60.4 | Horseshoe crab meal ..... | 61.5 |
| Spoiled menhaden meal ..... | 65.5 | Spoiled horseshoe crab meal ..... | 67.7 |

1/Made from lean marine groundfish fillet scrap.

Pilchard, "whitefish," and menhaden meals were tested by separate inclusion in the diet. Half of each sample was spoiled under conditions of high heat and

Table 2 - Data on Mean Food Intake and Mean Gain in Live Weight of Rats Fed for a 60-day Period with Diets Containing about 30 Percent Fish Meal

| Diet Designation | Number of Rats | Mean Food Intake | Mean Gain in Live weight | Estimated Mean Gain in Live Weight[1] |
|---|---|---|---|---|
| Meal | | Grams | Grams | Grams |
| Commercial pilchard ..... | 9 | 581.8 | 165.0 | 172.8 |
| Spoiled pilchard ..... | 9 | 607.4 | 155.4 | 152.6 |
| Commercial menhaden ..... | 9 | 521.9 | 136.3 | 169.0 |
| Spoiled menhaden ..... | 10 | 633.9 | 167.3 | 153.5 |
| Commercial "whitefish" ..... | 9 | 638.1 | 172.0 | 156.4 |
| Spoiled "whitefish" ..... | 9 | 616.8 | 167.8 | 161.1 |

1/Estimated mean gain in live weight for equal food intake (Y-bx where b = 0.4157). Snedecor (1940).

humidity, by being first mixed with water to a thick paste, and then held at about 120° F. for one week in large shallow trays. The spoiled meals had extremely foul odors. The decomposition was purposely permitted to become more extensive than would occur under any but the most adverse storage conditions, in order to accentuate any alteration in the meal. The meals were then dried at a temperature under 120° F., finely ground, and stored in covered metal cans. (See Table 1, p. 2.)

Table 3 - Analysis of Covariance, and Test of Significance of Adjusted Mean Gain in Live Weight of Groups Reported in Table 2

| Source of Variation | Degree of Freedom | SUM OF SQUARES AND PRODUCTS | | | ERRORS OF ESTIMATE | | |
|---|---|---|---|---|---|---|---|
| | | $Sx^2$ | Sxy | $Sy^2$ | Sums of Squares | Degrees of Freedom | Mean Square |
| Total | 54 | 284,707.20 | 106,079.80 | 60,927.93 | 21,403.40 | 53 | |
| Groups | 5 | 85,477.18 | 23,252.39 | 7,794.05 | 2,703.99 | 5 | 540.80 |
| Within groups | 49 | 199,230.02 | 82,827.41 | 53,133.88 | 18,699.41 | 48 | 389.57 |
| | | | | F = 1.39 which is non-significant | | | |

X = food intake Y = gain in live weight

All animals were in excellent health at the close of the 60-day experimental period. The data in Tables 2 and 3 show that there were no statistically significant differences in the estimated mean gain in live weight between groups fed the various fish meals. At the levels fed, one meal was as satisfactory as another.

## Chick Feeding Experiment

### Allotment and Equipment

Cross bred New Hampshire-Barred Plymouth Rock or Barred Plymouth Rock chicks were used in the chick feeding experiments. They were obtained from a local hatchery when one day old, and were allotted into groups having approximately equal mean weights, and with similar ranges in the weights of individual chicks. No record was kept of the initial or final distribution of sex. All chicks were individually banded.

Water was kept before them at all times. On the second day, the experimental diets were available for 15-minute periods each during the forenoon and afternoon. From the third day to the end of the experimental period, usually six weeks in duration, the chicks had access to food at all times. The chicks were housed in a battery cage in groups. The cages were located in a room in which the temperature was maintained close to 80° F. Each cage was also fitted with brooder facilities.

### Diets Used

The diet consisted of a variable quantity of fish meal or protein supplement; sucrose, 15; lard, 5; salt mixture, U.S.P. XI No. 2, 2; agar, 2; dried brewer's yeast, 2; wheat germ, 1; cod liver oil, 2; and dextrin to make 100 parts by weight. Separate vitamin supplementation was also made.

The quantity of fish meal, or protein supplement fed varied for each series of experiments as follows:

Series 1 - 35 percent fish meal
" 2 - 30 " " "
Series 3 - 18 percent protein equivalent
" 4 to 10 - 22 " " "

For series 5 to 10, inclusive, all diets contained an additional 100 parts per million of manganese as $MnSO_4.4H_2O$. Manganese was added as a prophylactic against perosis. For series 6 to 10, inclusive, three percent soy-bean oil was

included in the diets at the expense of two percent lard and one percent dextrin. The soy-bean oil was added to supply vitamin K and the antiencephalomalacia and anti-gizzard erosion factors.

Chicks in series 1 to 6, inclusive, received 0.12 mg. thiamine hydrochloride, and 1.2 mg. crystalline riboflavin per kg. of diet. Those in series 7 to 10 received 0.36 mg. thiamine, and 1.2 mg. riboflavin per kg. diet. There were some exceptions which are noted in appropriate tables of data. The other dietary additions were made at the expense of an equivalent weight of dextrin.

In series 7 to 10, inclusive, a second control group was used in which the chicks were fed a commercial growing mash type of diet. The diet consisted of pilchard meal (about 65 percent protein), 7.5; meat scrap (about 50 percent protein), 7.5; dried skim milk, 5.0; ground yellow corn, 30.0; ground barley, 11.5; ground wheat, 10.0; wheat bran, 16.0; alfalfa meal, 7.5; ground limestone or oyster shell, 2.0; steamed bone meal, 1.0; salt, 1.0; and fish oil (containing 100 international units of vitamin D per gram), 1.0 to equal 100 parts by weight (Almquist, Jukes, and Newlon, 1938). The ration analyzed about 19.0 percent crude protein.

### Data for Lactalbumin

Lactalbumin was selected as a reference protein after the first preliminary feeding studies had been completed. It is rated as being very high in nutritive quality, and is probably only partially deficient in argenine content. The chicks fed the diet containing lactalbumin should have grown to the maximum limit permitted by an adequate protein source.

The chicks fed this basal diet and certain modified diets did not grow well, and there was high mortality (Table 4). Even replacement of ten percent of the

Table 4 - Data on Gain in Live Weight and Food Consumption of Survivors, and on Mortality of Chicks Fed Lactalbumin as Principal Source of Protein

| Diet Designation | Series | Group | Number of Chicks | | Mean Life | Mean Food Intake | Mean Gain in Live Weight | Food per Gram Gain |
|---|---|---|---|---|---|---|---|---|
| | | | Initial | Final | Days | Grams | Grams | Grams |
| Lactalbumin: | | | | | | | | |
| No addition[1] | 3 | 4 | 10 | 4 | 30.5 | 189.3 | 20.8 | 9.10 |
| Plus manganese[2] | 5 | 6 | 8 | 6 | 36.3 | 393.9 | 113.5 | 3.47 |
| Manganese and one percent argenine[2] | 5 | 11 | 8 | 4 | 35.3 | 376.1 | 120.0 | 3.13 |
| Plus soy-bean oil | 6 | 9 | 7 | 0 | 12.9 | | | |
| Replacement of 10 percent lactalbumin with equivalent weight of spoiled pilchard meal[3] | 6 | 10 | 7 | 4 | 31.3 | 352.0 | 111.8 | 3.15 |

1/ 35-day test, all others were 42-day tests.
2/ 0.36 mg. thiamine and 3.6 mg. riboflavin per kg. diet.
3/ One chick killed accidentally.

lactalbumin with an equivalent weight of spoiled pilchard meal did not promote better growth. No further studies were carried out with this source of protein since it was obvious that considerable experimental work would be needed to determine the inherent deficiencies.

### Data for Menhaden and "Whitefish" Meals

There was no difference in the growth rate or health of rats fed the different commercial or spoiled meals. This was not true for chicks (Table 5). Series 1

chicks, for some unexplainable reason, grew very much better when fed the "no addition" diets containing the commercial meals than did those in subsequent series. The chicks suffered, however, from severe perosis. Also the mortality was very high with series 4 chicks.

Table 5 - Data on Gain in Live Weight and Food Consumption of Survivors, and on Mortality of Chicks Fed Menhaden, and "Whitefish" Meals as Principal Source of Protein For a 6-Week Period

| Diet Designation | Series | Group | Number of Chicks | | Mean Life | Mean Food Intake | Mean Gain in Live Weight | Food per Gram Gain |
|---|---|---|---|---|---|---|---|---|
| | | | Initial | Final | Days | Grams | Grams | Grams |
| Commercial Menhaden Meal: | | | | | | | | |
| No addition | 1 | 3 | 9 | 8 | 41.8 | 921.2 | 300.6 | 3.06 |
| " " | 4 | 5 | 8 | 1 | 30.4 | 277.5 | 73.0 | 3.80 |
| | | Summary | 17 | 9 | 36.4 | 849.8 | 275.3 | 3.09 |
| Plus manganese | 4 | 11 | 8 | 0 | 18.9 | | | |
| Spoiled Menhaden: | | | | | | | | |
| No addition | 1 | 4 | 9 | 9 | 42.0 | 706.6 | 298.4 | 2.37 |
| " " | 2 | 3 | 9 | 7 | 38.1 | 1045.6 | 371.6 | 2.81 |
| " " | 4 | 6 | 8 | 4 | 33.5 | 607.9 | 274.0 | 2.22 |
| | | Summary | 26 | 20 | 38.0 | 805.5 | 319.2 | 2.52 |
| Plus manganese | 2 | 4 | 9 | 8 | 39.7 | 1024.6 | 325.3 | 3.15 |
| " " | 4 | 12 | 8 | 5 | 34.8 | 832.3 | 369.0 | 2.26 |
| | | Summary | 17 | 13 | 37.4 | 950.7 | 342.1 | 2.78 |
| Commercial "Whitefish" Meal: | | | | | | | | |
| No addition | 1 | 5 | 8 | 5 | 34.6 | 523.8 | 174.0 | 3.01 |
| " " | 4 | 3 | 8 | 0 | 21.1 | | | |
| | | Summary | 16 | 5 | 27.9 | 523.8 | 174.0 | 3.01 |
| Plus manganese | 4 | 9 | 8 | 1 | 28.1 | 399.7 | 124.0 | 3.22 |
| Spoiled "Whitefish" Meal: | | | | | | | | |
| No addition | 1 | 6 | 8 | 7 | 37.6 | 644.6 | 279.4 | 2.31 |
| " " | 4 | 4 | 8 | 3 | 33.9 | 541.3 | 291.3 | 1.86 |
| | | Summary | 16 | 10 | 35.8 | 613.6 | 283.0 | 2.17 |
| Plus manganese | 4 | 10 | 8 | 8 | 42.0 | 674.8 | 304.1 | 2.22 |

In general, the chicks fed the spoiled menhaden or "whitefish" meals grew to a larger size, and required less food per unit gain in live weight than did those fed the commercial meals. The data indicate that one or more factors produced during spoilage at least partially alleviated the deficiency symptoms. There was no evidence of toxicosis when spoiled meals were fed.

### Vitamin K Deficiency Study

It was noted that chicks fed the diet containing commercial fish meal had subcutaneous hemorrhages. About this time, some of the early work was published on reduction of blood clotting time with extracts prepared from fish meal or alfalfa meal. Blood clotting time determined with the glass capillary method varied from 2 to 24 minutes, with an average of 10 minutes, for 11 Leghorn chicks fed for one month after hatching with the standard diet containing 18 percent protein from commercial menhaden meal.

A petroleum ether extract was prepared from spoiled menhaden meal according to the method reported by Osterberg (1938). Another group of chicks were fed the beforementioned diet supplemented with 30 mg. of extract per 100 g. of diet. Thirteen chicks fed this diet had a blood clotting time of three minutes or less, and mostly less than two minutes.

At first, it was believed that the vitamin K deficiency, as indicated by the prolonged blood clotting time, was responsible for poor growth. This premise was

not necessarily correct because three chicks fed commercial pilchard meal had blood clotting times of 13, over 30, and over 46 minutes, and gained in live weight, respectively, 247, 244, and 124 g. (Table 7, series 5, group 13). Four chicks fed commercial pilchard meal plus three percent soy-bean oil had blood clotting times of 1, 2, 3, and 3 minutes, and gained in live weight, respectively, 163, 178, 127, and 249 g. (Table 7, series 5, group 2). Also three chicks fed spoiled horseshoe crab meal had blood clotting times of 1, 1, and 2 minutes, and gained in live weight, respectively, 139, 80, and 76 g. (Table 6, series 5, group 7).

Table 6 - Data on Gain in Live Weight and Food Consumption of Survivors and on Mortality of Chicks Fed Horseshoe Crab Meal as Principal Source of Protein[1]

| Diet Designation | Series | Group | Number of Chicks Initial | Number of Chicks Final | Mean Life | Mean Food Intake | Mean Gain in Live Weight | Food per Gram Gain |
|---|---|---|---|---|---|---|---|---|
| | | | | | Days | Grams | Grams | Grams |
| 42 DAY TEST | | | | | | | | |
| Horseshoe Crab Meal: | | | | | | | | |
| No addition | 2 | 1 | 9 | 9 | 42.0 | 420.8 | 90.7 | 4.64 |
| Plus manganese | 5 | 5 | 8 | 7 | 37.5 | 723.8 | 212.4 | 3.41 |
| " " | 5 | 8 | 8 | 6 | 35.1 | 507.3 | 171.5 | 2.96 |
| | Summary | | 16 | 13 | 36.3 | 623.9 | 193.5 | 3.22 |
| Plus soy-bean oil[2] | 5 | 9 | 8 | 6 | 33.9 | 572.8 | 165.2 | 3.47 |
| Plus one percent argenine[2] | 5 | 10 | 7 | 3 | 24.7 | 620.1 | 197.7 | 3.14 |
| Spoiled Horseshoe Crab Meal: | | | | | | | | |
| No addition | 2 | 2 | 9 | 6 | 37.2 | 378.0 | 45.7 | 8.27 |
| Plus manganese | 5 | 7 | 8 | 7 | 39.3 | 444.9 | 99.6 | 4.47 |
| Plus soy-bean oil, one percent argenine, and two percent tricalcium phosphate[2] | 5 | 12 | 7 | 5 | 35.4 | 697.3 | 237.2 | 2.94 |
| 35 DAY TEST | | | | | | | | |
| Horseshoe Crab Meal: | | | | | | | | |
| No addition | 3 | 1 | 10 | 8 | 34.0 | 201.9 | 31.4 | 6.43 |
| Half protein replaced with lactalbumin | 3 | 2 | 10 | 6 | 31.8 | 170.2 | 25.8 | 6.60 |
| Plus alfalfa meal | 3 | 3 | 10 | 8 | 34.4 | 209.5 | 33.4 | 6.27 |
| 31 DAY TEST | | | | | | | | |
| Spoiled Horseshoe Crab Meal: | | | | | | | | |
| No addition | 3 | 5 | 10 | 3 | 24.3 | 179.6 | 17.3 | 10.38 |
| Half protein replaced with lactalbumin | 3 | 6 | 10 | 6 | 28.4 | 180.4 | 23.4 | 7.71 |
| Plus alfalfa meal[3] | 3 | 7 | 10 | 3 | 28.0 | 132.0 | 16.0 | 8.25 |

1/Also called King crabs (Limulus). The crude protein content of horseshoe crab meal was 61.5 percent, and for spoiled meal was 67.7 percent (N x 6.25).

2/0.35 mg. thiamine, and 3.5 mg. riboflavin, instead of 0.12 mg. thiamine and 1.2 mg. riboflavin per kg. diet.

3/Eight percent alfalfa meal, and enough horseshoe crab meal to make 18 percent protein.

Since the object of the experiment was to identify deficiency factors, rather than the extent of the deficiency, all chicks in series 6 to 10 were fed soy-bean oil at a level sufficient to furnish ample quantities of vitamin K, and the anti-encephalomalacia factor and the anti-gizzard erosion factor.

### Data for Horseshoe Crab Meal

Two samples of horseshoe crab meal were obtained which had been produced under pilot plant conditions. There seemed to be no difference between the meals. Chicks in series 2 and 3, and series 5, group 8, were fed sample 1, and the remainder, sample 2 (Table 6). The series 3 chicks, which were fed horseshoe crab meal, except for group 4, which was fed lactalbumin (Table 4), grew so poorly that they had to be destroyed in 31 or 35 days.

Table 7 - Data on Gain in Live Weight and Food Consumption of Survivors and on Mortality of Chicks Fed Commercial and Spoiled Pilchard Meal, as a Principal Source of Protein, and a Commercial Type of Growing Mash for a 6-Week Period

| Diet Designation | Series | Group | Number of Chicks Initial | Number of Chicks Final | Mean Life | Mean Food Intake | Mean Gain in Live Weight | Food per Gram Gain |
|---|---|---|---|---|---|---|---|---|
| | | | | | Days | Grams | Grams | Grams |
| Growing Mash | 6 | 2 | 10 | 7 | 31.4 | 950.1 | 340.4 | 2.79 |
| " " | 7 | 9 | 10 | 8 | 34.6 | 1151.9 | 361.9 | 3.18 |
| " " | 8 | 12 | 10 | 9 | 38.7 | 1023.1 | 373.0 | 2.74 |
| " " | 9 | 4 | 10 | 10 | 42.0 | 1094.8 | 451.8 | 2.42 |
| " " | 10 | 4 | 11 | 11 | 42.0 | 1156.1 | 484.0 | 2.39 |
| | Summary | | 51 | 45 | 37.8 | 1083.1 | 410.6 | 2.64 |
| Commercial Pilchard Meal: | | | | | | | | |
| No addition | 1 | 1 | 9 | 2 | 24.8 | 565.8 | 205.5 | 2.75 |
| " " | 4 | 1 | 8 | 2 | 25.5 | 477.6 | 137.0 | 3.49 |
| | Summary | | 17 | 4 | 25.1 | 521.8 | 171.3 | 3.05 |
| Plus manganese | 4 | 7 | 8 | 0 | 26.8 | | | |
| " " | 5 | 1 | 8 | 0 | 19.4 | | | |
| " " 1/ | 5 | 13 | 8 | 4 | 30.8 | 568.1 | 197.5 | 2.88 |
| | Summary | | 24 | 4 | 25.6 | 568.1 | 197.5 | 2.88 |
| Plus soy-bean oil | 5 | 2 | 7 | 4 | 34.9 | 470.2 | 179.3 | 2.62 |
| " " " | 6 | 1 | 10 | 0 | 22.2 | | | |
| | Summary | | 17 | 4 | 27.4 | 470.2 | 179.3 | 2.62 |
| Higher level thiamine | 7 | 8 | 10 | 8 | 38.8 | 707.0 | 305.6 | 2.31 |
| " " | 8 | 1 | 10 | 9 | 40.3 | 622.6 | 258.0 | 2.41 |
| " " | 9 | 3 | 11 | 10 | 40.8 | 676.6 | 292.2 | 2.32 |
| " " | 10 | 1 | 11 | 3 | 27.3 | 386.9 | 137.0 | 2.82 |
| " " | 10 | 10 | 10 | 6 | 32.4 | 538.0 | 121.2 | 4.44 |
| | Summary | | 52 | 36 | 35.8 | 622.6 | 245.2 | 2.54 |
| Spoiled Pilchard Meal: | | | | | | | | |
| No addition | 1 | 2 | 9 | 8 | 41.9 | 764.6 | 329.6 | 2.31 |
| " " | 4 | 2 | 8 | 2 | 34.8 | 476.3 | 112.5 | 4.23 |
| | Summary | | 17 | 10 | 38.5 | 707.0 | 286.2 | 2.47 |
| Plus manganese | 4 | 8 | 8 | 6 | 37.0 | 664.6 | 260.7 | 2.55 |
| Commercial Pilchard Meal: | | | | | | | | |
| Five percent of meal used was spoiled 2/ | 5 | 3 | 8 | 4 | 30.1 | 844.0 | 374.8 | 2.25 |
| Twenty-five percent of meal used was spoiled 2/ | 5 | 4 | 8 | 7 | 39.9 | 921.6 | 389.1 | 2.37 |
| Ten percent of meal used was spoiled | 7 | 10 | 10 | 10 | 42.0 | 1034.3 | 506.9 | 2.04 |
| " " " " " " " | 8 | 6 | 10 | 8 | 36.6 | 883.1 | 422.1 | 2.09 |
| " " " " " " " | 9 | 5 | 10 | 9 | 38.7 | 887.6 | 478.1 | 1.86 |
| " " " " " " " | 10 | 5 | 10 | 10 | 42.0 | 923.1 | 465.4 | 1.98 |
| | Summary | | 40 | 37 | 39.8 | 932.0 | 468.1 | 1.99 |
| Plus soy-bean oil, and water extract equivalent to 10 percent spoiled meal | 6 | 4 | 10 | 6 | 32.6 | 610.3 | 280.5 | 2.18 |
| Water extract equivalent to 10 percent spoiled menhaden meal | 7 | 14 | 10 | 7 | 35.3 | 709.8 | 401.0 | 1.77 |
| Water extract equivalent to 10 percent spoiled pilchard meal (higher level thiamine) | 9 | 6 | 10 | 10 | 42.0 | 970.7 | 567.7 | 1.71 |
| Water extract equivalent to 10 percent spoiled pilchard meal (higher level thiamine) | 10 | 6 | 10 | 10 | 42.0 | 920.1 | 476.2 | 1.93 |
| | Summary | | 20 | 20 | 42.0 | 945.4 | 522.0 | 1.81 |
| Same as above plus 10 mg. pantothenic acid per 100 g. diet | 10 | 8 | 11 | 10 | 38.6 | 1026.2 | 523.2 | 1.96 |

1/ A second sample of pilchard meal.

2/ 0.36 mg. thiamine and 3.6 mg. riboflavin per kg. diet. This level of thiamine and riboflavin was also fed to all groups of series 7 to 10.

None of the chicks fed horseshoe crab meal grew satisfactorily, and the results were so uniformly poor that no further experimental work was conducted with this meal.

### Data for Commercial and Spoiled Pilchard Meals

The chicks fed the commercial pilchard meal, unsupplemented or with added manganese or soy-bean oil did not grow well, and there was a high rate of mortality (Table 7). The threefold increases in the level of thiamine fed to these groups helped considerably in reducing mortality in most instances but did not materially affect growth rate.

Feeding spoiled pilchard meal helped some, and particularly when ten percent spoiled meal was substituted for an equivalent weight of commercial meal in the series in which the chicks received diets supplemented with manganese, soy-bean oil, and the higher level of thiamine. Chicks which received all of the supplements grew as well and with as economical use of food as those fed the growing mash.

For the first time, therefore, satisfactory growth was obtained with the experimental basal diet using mostly commercial meal as a source of protein. The spoiled meal was the source of one or more factors that permitted satisfactory growth. The meals were then extracted with various solvents to determine whether the factors were vitamin or protein in nature.

For all experiments, the spoiled meal was prepared by the method explained in the section on the rat feeding experiment. A crude water extract was prepared when this meal was stirred with warm water, about 120° F., allowed to settle, and the liquor was decanted. The extraction was repeated three or four times, the total quantity of water equalling to 8 to 10 times the weight of meal. This extract was centrifuged and the clear liquor was decanted off and concentrated under vacuum at a temperature not exceeding 130° F. to 10 percent total solids. This concentrate was mixed with dextrin, and dried at room temperature. The extract contained only about 0.7 percent of the original nitrogen of the meal.

Extract equivalent to the 10 percent, by weight, of spoiled meal was incorporated into the diet containing commercial pilchard meal. The chicks fed the water extract grew very well, and the mortality was low. These results indicated that quality of protein was not a limiting factor. The good results seemed to be due to some water soluble fraction, probably one or more of the water soluble vitamins.

### Fractionation Studies of Water Extract

The water extract of spoiled meal was subjected to a series of fractionations in order to determine whether the growth-promoting factors could be separated or concentrated. The data in Tables 8 and 9 indicate that the untreated water extract and that autoclaved for one hour at pH 8.5 and 15 lbs. pressure, permitted a mean gain in live weight for the group that was significantly better than the over-all mean. One of the two control groups fed 10 percent spoiled meal (series 7, group 10) also made superior gains. These data indicate that the factor is not heat labile during a rather short period under mild conditions.

On the other hand, the addition to the diet of products extractable with fat solvents, diethyl ether and ethyl alcohol, permitted only very poor growth with an even higher rate of mortality than when the unsupplemented diet containing only commercial pilchard meal was fed. The sought-for factor or factors definitely could not be extracted with these solvents.

Fuller's earth apparently was a poor adsorbent for the active material. The chicks fed either the fuller's earth before elution or two different elution products did not grow well. The mortality was also very high when the uneluted fuller's earth was fed. These data indicate that the chicks were unable to utilize what little material was adsorbed on the fuller's earth.

Table 8 - Data on Gain in Live Weight and Food Consumption of Survivors and on Mortality of Chicks Fed for a 6-Week Period on Various Extracts Made From Spoiled Pilchard Meal, and Added to a Diet in which Commercial Pilchard Meal was the Principal Source of Protein

| Diet Designation | Series | Group | Number of Chicks | | Mean Life | Mean Food Intake | Mean Gain in Live Weight | Food per Gram Gain |
|---|---|---|---|---|---|---|---|---|
| | | | Initial | Final | Days | Grams | Grams | Grams |
| Water extract, total | 7 | 1 | 10 | 9 | 41.7 | 847.5 | 490.6[1] | 1.73 |
| Ether extract | 7 | 2 | 10 | 6 | 32.2 | 673.1 | 302.0[1] | 2.23 |
| Water extract, pH 8.5 and autoclaved at 15 lbs. for one hr. | 7 | 3 | 10 | 6 | 33.9 | 1021.2 | 499.2[1] | 2.05 |
| Fullers' earth filtrate, pH 5 | 7 | 4 | 10 | 8 | 35.5 | 874.4 | 450.1 | 1.94 |
| Fullers' earth eluted with barium hydroxide[2] | 7 | 5 | 10 | 9 | 38.9 | 743.9 | 315.9 | 2.35 |
| Fullers' earth eluted with pyridine-methanol[3] | 7 | 6 | 10 | 8 | 40.5 | 737.7 | 345.0 | 2.14 |
| Fullers' earth before elution | 7 | 7 | 10 | 5 | 32.8 | 680.2 | 279.6[1] | 2.43 |
| Water extract, concentrated under vacuum under 50° C. | 8 | 5 | 10 | 10 | 42.0 | 800.0 | 412.8 | 1.94 |
| Water extract, pH 11 with NaOH and refluxed 3 hrs. | 8 | 3 | 10 | 2 | 17.8[1] | 847.5 | 321.5 | 2.64 |
| Water extract, pH 1 with HCl, fullers' earth liquid pH 7 and concentrated | 8 | 4 | 10 | 7 | 34.5 | 951.1 | 430.4 | 2.21 |
| Water extract, ethyl alcohol solids | 8 | 13 | 10 | 6 | 30.6[1] | 525.8 | 218.8[1] | 2.40 |
| Water extract, ethyl alcohol washings | 8 | 2 | 10 | 8 | 36.7 | 812.1 | 402.5 | 2.02 |

1/ A statistically significant difference at 5 percent level from means used in data in Table 9. Over-all mean gain in live weight is 381.13 g. and over-all mean length of life is 35.93 days.

2/ Two-tenths normal barium hydroxide was used. The barium was precipitated with sulfuric acid, and filtrate was fed.

3/ A mixture of 1:1:4 pyridine, methanol, and water. The pyridine was removed and the filtrate was fed.

Table 9 - Analysis of Variance for Data on Gain in Live Weight and Length of Life for Groups Reported in Table 8 and also Control Groups[1] in series 7 and 8

| Source of variation | Degrees of Freedom | Sums of Squares | Mean Square |
|---|---|---|---|
| Gain in live weight: | | | |
| Total | 118 | 1,724,477.1 | |
| Groups | 15 | 916,836.9 | 61,122.46 |
| Within groups | 103 | 807,640.2 | 7,841.17 |
| | F = 7.80 which is highly significant | | |
| Days length of life: | | | |
| Total | 159 | 21,472.1 | |
| Groups | 15 | 5,518.7 | 367.91 |
| Within groups | 144 | 15,953.4 | 110.79 |
| | F = 3.32 which is highly significant | | |

1/ Series 7, group 8, and series 8, group 1, fed commercial pilchard meal. Series 7, group 10, and series 8, group 6, fed 10 percent spoiled meal (Table 7).

The active material was also definitely destroyed when refluxed with alkali. This is a rather drastic treatment, and would destroy most known water soluble vitamins. The chicks fed the alkali refluxed extract grew less well than the poorest of the two groups fed commercial meal alone (series 8, group 1).

Table 10 - Data on Gain in Live Weight and Food Consumption of Survivors and on Mortality of Chicks Fed for a 6-Week Period on a Diet Containing Commercial Pilchard Meal as Principal Source of Protein to Which Were Added Various Supplements

| Diet Designation | Series | Group | Number of Chicks Initial | Number of Chicks Final | Mean Life | Mean Food Intake | Mean Gain in Live Weight | Food per Gram Gain |
|---|---|---|---|---|---|---|---|---|
| | | | | | Days | Grams | Grams | Grams |
| Plus soy-bean oil: | | | | | | | | |
| Three percent dried brewer's yeast[1] | 6 | 5 | 10 | 2 | 20.7 | 399.5 | 151.5 | 2.64 |
| Five percent alfalfa meal | 6 | 11 | 10 | 1 | 20.5 | 591.4 | 257.0 | 2.30 |
| One percent wheat germ oil | 6 | 7 | 10 | 3 | 22.6 | 516.2 | 234.3 | 2.20 |
| One percent liver extract[2] | 6 | 6 | 10 | 0 | 12.4 | | | |
| Nicotinic acid, 5 mg. per 100 g. | 6 | 8 | 10 | 1 | 10.0 | 455.3 | 171.0 | 2.66 |
| Plus added thiamine: | | | | | | | | |
| Ten percent autoclaved peanuts[3] | 7 | 13 | 10 | 9 | 41.0 | 786.2 | 403.8 | 2.17 |
| Five percent dried kelp meal | 7 | 15 | 10 | 9 | 41.4 | 832.2 | 428.9 | 1.94 |
| One percent rice bran concentrate[4] | 7 | 11 | 10 | 10 | 42.0 | 1002.9 | 448.4 | 2.24 |
| One percent rice bran concentrate | 8 | 11 | 10 | 9 | 41.1 | 773.5 | 398.6 | 1.94 |
| | Summary | | 20 | 19 | 41.6 | 888.2 | 403.6 | 2.20 |
| Three percent rice bran concentrate | 8 | 9 | 10 | 8 | 36.1 | 885.8 | 453.1 | 1.95 |
| " " " " | 9 | 8 | 10 | 10 | 42.0 | 1005.0 | 531.7 | 1.89 |
| | Summary | | 20 | 18 | 39.1 | 945.4 | 492.4 | 1.92 |
| One percent liver extract | 7 | 12 | 10 | 5 | 31.0 | 998.7 | 460.6 | 2.17 |
| Two " " " | 8 | 7 | 10 | 10 | 42.0 | 927.6 | 467.1 | 2.00 |
| " " " " | 9 | 2 | 10 | 10 | 42.0 | 1009.7 | 560.2 | 1.80 |
| " " " " | 10 | 7 | 10 | 8 | 37.6 | 988.4 | 547.5 | 1.81 |
| | Summary | | 30 | 28 | 40.5 | 975.2 | 524.9 | 1.86 |
| Pyridoxine, 0.3 mg. per 100 g. | 8 | 8 | 10 | 6 | 31.6 | 661.6 | 263.7 | 2.51 |
| Pyridoxine, 0.6 mg. per 100 g. | 8 | 10 | 10 | 6 | 33.5 | 816.5 | 240.2 | 3.40 |
| Five percent chondroitin | 9 | 7 | 10 | 8 | 36.8 | 631.5 | 221.5 | 2.85 |
| p-aminobenzoic acid, 0.05 g. per 100 g. | 10 | 9 | 11 | 6 | 34.0 | 544.7 | 206.5 | 2.64 |
| Pantothenic acid, 10 mg. per 100 g. | 9 | 1 | 11 | 11 | 42.0 | 883.8 | 528.4 | 1.67 |
| Pantothenic acid, 10 mg. per 100 g. | 10 | 2 | 10 | 8 | 37.8 | 741.2 | 378.9 | 1.96 |
| | Summary | | 21 | 19 | 39.9 | 812.5 | 453.7 | 1.79 |
| Pantothenic acid, 20 mg. per 100 g. | 10 | 3 | 10 | 8 | 39.7 | 808.7 | 415.0 | 1.95 |

1/Anheuser-Busch Inc., strain K yeast.
2/Eli Lilly and Company liver extract Lilly.
3/Raw peanuts were autoclaved for five hours at 15 pounds pressure.
4/National Oil Products Co., Harrison, N. J. Vitab type II concentrated aqueous extract of rice bran.

It was not possible to make a covariance analysis of gain in live weight and food consumption since the chicks were fed by groups instead of as individuals. In general, the groups which grew the best required the least food per unit gain in live weight. Considerable reliance, in this instance, can be placed on the

estimate of significance of differences by analysis of variance for gain in live weight, since the group means which were significantly better or poorer than the over-all mean can be correlated with the fractionation study.

The active material was resistant to a reasonable degree of heat, and to a fairly strong acid. The active material was probably neither an amino acid or a protein, since so little nitrogen was found in the water extract. A mineral element was probably not involved because the active material was destroyed when refluxed with alkali. The growth-promoting activity was apparently due to one or more water-soluble organic compounds.

### Data on Supplementation Studies

The fractionation studies reported in the preceding section indicated that the active material consisted of one or more organic compounds. Rather than to isolate and identify these, a series of feeding studies were conducted in which the basal commercial pilchard meal diet was supplemented with concentrates or pure vitamins.

The data in Table 10 indicate that the higher level of thiamine was essential to satisfactory growth. This interpretation may have to be modified somewhat since series 6 chicks did not grow as well as those from several other series. The three percent brewer's yeast should have been a good source of thiamine.

Pyridoxine, chondroitin, p-aminobenzoic acid were not helpful. The added nicotinic acid also probably was not needed. Autoclaved peanuts and dried kelp meal both contained the active material to a reasonable degree. It is, however, not possible without repetition of experiments to come to any conclusion on the value of either the yeast or alfalfa meal.

Table 11 - Analysis of Variance for Data on Gain in Live Weight of Selected Groups of Chicks Receiving Diet Containing Commercial Pilchard Meal Plus Supplements

| Source of variation | Degrees of Freedom | Sums of Squares | Mean Square |
|---|---|---|---|
| Total | 121 | 1,226,460.8 | 19,011.43 |
| Groups | 4 | 76,045.7 | 9,832.61 |
| Within groups | 117 | 1,150,415.1 | |
| | F = 1.93 which is not significant | | |

Note: Over-all mean gain in live weight = 494.10 g.
Thirty-seven surviving chicks fed 10 percent spoiled meal, 20 fed equivalent water extract, 18 fed 3 percent rice bran concentrate, 28 fed 2 percent liver extract, and 19 fed 10 mg. pantothenic acid of per 100 g. of diet (Tables 7 and 10).

Pantothenic acid, liver extract, and rice-bran concentrate were the best supplements. The data in Table 11 show that growth obtained with these supplements was not significantly different from that obtained with spoiled pilchard meal or with the water extract of spoiled meal.

## DISCUSSION

The basal diet that was used is not commercially practical, but it did permit feeding large quantities of fish meal. Except for horseshoe crab meal, the meals used apparently supplied sufficient high quality protein to promote satisfactory growth. Furthermore, experimental spoilage of meal under conditions of high temperature and humidity, and under an aerobic environment, did not produce any toxic products.

On the other hand, growth-promoting factors in addition to such accessory factors as vitamin K were synthesized by bacteria and molds during the experimental

spoilage. These factors were extractable with water and could withstand enough heat so the water extract could be concentrated and dried on dextrin. No attempts were made to isolate and identify these compounds.

Further studies showed that rice-bran concentrate, liver extract, and pantothenic acid when added singly to the commercial pilchard meal diet, did permit equivalent growth to that produced when one-tenth of the commercial meal was replaced by spoiled meal, or when the diet was supplemented with an equivalent amount of the water soluble extract of the spoiled meal. Whether pantothenic acid is the only vitamin involved cannot be determined from these studies. From the practical standpoint, further work along these lines seems hardly worthwhile. Nor is an extensive review of literature indicated to correlate the results, since experimental diets and conditions vary widely, and the data are difficult to interpret in terms of the requirements for essential food elements, especially for vitamins.

At the close of the experimental period, all of the chicks of sufficient size were killed, skinned in most instances, and dressed. These were distributed to various staff members who had them cooked and taste-tested at their homes. No fishy or other off flavors were reported, except for a few chicks which were not allotted to any single group. For most groups, no chicks were reported as having fishy or other off flavors. Some randomly selected birds were plucked rather than skinned. The skin or subcutaneous fat did not have any off flavor unless it was present in the flesh.

## CONCLUSIONS

1. The commercial fish meals which were fed are an excellent source of protein, and can be incorporated in rather large amounts, if necessary, into a properly balanced diet without deleterious effects.

2. Experimental spoilage under conditions of high heat and humidity, and in an aerobic environment, does not produce toxic products.

3. Water-soluble growth factors and vitamin K are synthesized by bacteria and molds during the experimental spoilage.

4. In only a few instances will fishy or other off flavors be detected in the flesh of chicks fed comparatively large quantities of commercial or spoiled fish meal during a six-week period.

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