U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Wildlife Services

Technical Papers
OF THE BUREAU OF SPORT FISHERIES AND WILDLIFE

13. Test of Different Components in the Abernathy Salmon Diet

By Laurie G. Fowler and Joe L. Banks

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Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
Washington, D.C. • August 1967

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402 – Price 20 cents

CONTENTS

TEST OF DIFFERENT COMPONENTS IN THE ABERNATHY SALMON DIET

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ABSTRACT.--Substitute components in the Abernathy salmon diet were tested in 2 years of feeding trials with fall chinook salmon fingerlings (Oncorhyncus tshawytscha). These diet tests indicated that turbot meal and dogfish meal were adequate substitutes for salmon meal. Soybean oil was superior to peanut, corn, cottonseed, or safflower oil. Corn gluten, safflower, or soybean meals were inadequate substitutes for cottonseed meal. Dried buttermilk was equal to dried skim milk. The amount of dried skim milk or wheat germ meal could not be reduced without deleterious effects. The mixed diet could be stored under refrigeration for 3 days and the premixed meals could be held at room temperature for several weeks without observable deleterious effect on the fish; storage for greater periods was deleterious.

The Salmon-Cultural Laboratory has been engaged for several years in an experimental program to develop nutritionally adequate and economically feasible diets for the artificial propagation of salmon (Oncorhynchus). Results of previous feeding trials at this laboratory (Combs et al., 1962; Fowler et al., 1964, 1966) led to development of an all-meal diet which has maintained salmon for periods of more than 26 weeks. This all-meal diet, called the Abernathy salmon diet, is composed of salmon carcass meal, dried skim milk, cottonseed meal, wheat germ meal, a vitamin package, a binder, peanut oil, and water. In feeding trials in 1964 and 1965, reported on here, substitutions and alterations of the components of this diet were tested with chinook salmon fingerlings (O. tshawytscha). The objective was to develop an optimum combination of ingredients.

The 1964 feeding trials were designed to test the protein quality of several fish meals to be used as possible replacements for salmon carcass meal. The 1965 trials were directed toward testing variations and substitutions of the remaining basal components of the diet and testing methods of diet preparation.

Results of the trials indicated that several substitute ingredients were as good as or better than the original diet components and that some methods of diet preparation were deleterious while others were satisfactory.

METHODS AND TECHNIQUES

The methods and techniques for the 1964 and 1965 feeding trials were nearly identical and were similar to those developed by Burrows et al. (1951, 1952). Fish used were hatched from a single day's egg take. Samples of fish were withdrawn from this population and stocked randomly into 6-foot circular tanks at the rate of 500 grams per tank. Each diet was fed to duplicate tanks of fish for 24 weeks. The fish were weighed biweekly, and the amount of food was adjusted according to the weight of the fish. Well water was supplied at the rate of 7 gallons per minute to each tank and was at a constant temperature of 53° F.

The chemical composition of feeds was the basis for diet formulation, with caloric levels calculated on the basis of available calories as reported by Phillips and Brockway (1959).

The caloric values used for these calculations were 4.0 calories per gram of protein (3.9 in 1965), 8.0 calories per gram of fat (9.0 calories per gram of vegetable oil), and 1.6 calories per gram of carbohydrate. The level of protein in the experimental diets was maintained at 25 percent (wet weight basis) by partial reconstitution of the diet with water, according to the method developed by Phillips and Brockway (1959). All substitutions of test ingredients in the basal ration were on an isoprotein basis. The diets were maintained isocaloric by adjusting the vegetable-oil portion of the diet. Thiobarbituric acid values (TBA) of the diet ingredients were determined by the method developed by Yu and Sinnhuber (1957).

A crystalline vitamin package, corresponding to the recommended maximum levels for chinook salmon (Halver, 1957; Halver et al., 1960), was added daily to each experimental diet. The composition of this vitamin package is shown in table 1. During 1965 the choline portion of this package was eliminated except from one diet. The meat control diet fed in 1964 was bound by the addition of 2 grams of salt per 100 grams of diet. The experimental diets were bound by the addition of 2 grams of salt and 2 grams of high-viscosity, carboxymethyl cellulose (CMC) per 100 grams of diet. During 1965, salt was eliminated from the diets, and CMC was used exclusively. The vitamin package was mixed with CMC as a carrier, and after addition of the vitamins CMC was added until the vitamin package and the additional CMC made up 2 percent of the total diet fed. The addition was accompanied by a 2-percent reduction in the amount of water added to the diet. All diets were ricer fed. The chemical composition, as determined by proximate analysis, of 10 fish from each diet withdrawn after 12 and 24 weeks was one of the criteria used for analyzing the results of the experiments. Protein deposition, rather than total gain in weight, was the measure of growth. Protein utilization, a measure of the efficiency of the protein in the diet, was calculated by dividing the amount of protein fed by the amount of protein deposited in the fish. During the 1965 trials, when mortality was high, all dead fish were weighed and used in the calculation of protein deposition, protein utilization, and fat deposition. At the conclusion of the experiments the data were analyzed statistically by analysis of variance for paired experiments as outlined by Snedecor (1956). Significant differences were determined at the 5-percent confidence level.

Histological examinations of five fish taken randomly from key diets were made by Dr. Edward M. Wood at the termination of the feeding trials. During 1965, total blood cell counting of samples from 10 fish from each diet was done by a Model B Coulter Counter. Stamina tests of paired samples of 100 fish each from selected diets were run on the 1965 diet experiments. A performance rating determined by the formula described by Thomas, Burrows, and Chenoweth (1964) was used to measure the potential stamina of these samples.

RESULTS OF THE 1964 FEEDING TRIALS

The 21 diets fed during 1964 included a standard meat control and 20 experimental diets designed to test the protein quality of several fish meals. The fish meals were prepared from the following species: 1961 chinook salmon carcass; 1963 chinook salmon carcass; 1963 herring (*Clupea harengus pallasi*); 1963 turbot (*Atheresthes stomias*); 1963 dogfish (*Squalus acanthias*); 1963 rockfish (*Sebastodes* sp.); 1963 sole (*Microstomus pacificus*); and 1963 tuna (*Thunnus alalunga*). The identification of the sources of these meals is assumed to be correct, but may be in error since they were obtained from commerical producers. The chemical analyses of the meals and other ingredients used in the 1964 diets are presented in table 2. An outline of the variables of diet composition as well as a summary of the results of this experiment are presented in table 3.

Diet 3 and diets 11 through 16 were formulated initially using the 1963 salmon carcass meal. At the end of 4 weeks of feeding, the growth rate of these fish started to decline. This 1963 salmon meal had a significantly higher TBA value (80.0) than did the 1961 salmon carcass meal (25.0). The high level of rancidity as indicated by a TBA value of 80 was not lethal to the fish but did

Table 1:--Composition of vitamin package as fed per day per kilogram of fish.

	Milligrams
Thiamine	0.20
Riboflavin	1.00
Pyridoxine	0.43
Niacin	7.00
Pantothenic acid	2.00
Inositol	20.00
Biotin	0.04
Folic acid	0.15
Sub total	30.82
CMC	469.18
Total	500.00
Choline 1/	60.00

1/ Choline dissolved in water and added as a separate component.

reduce growth, suggesting a sublethal, toxic effect. In order not to obscure the growth potentials of the complementary fish meals in diets 11 through 16, the rancid 1963 salmon meal was replaced by the 1961 salmon meal. This meal had been used successfully as a diet component at this laboratory previously (Fowler et al., 1964, 1966), and was used as the control for evaluating the other fish meals tested in this experiment. Diet 3 was continued as originally formulated.

All 21 diets successfully maintained fish for the 24-week feeding period with insignificant mortality and without visible signs of nutritional deficiencies. With the exception of two, the diets tested produced fish with growth rates equal to or better than the meat control diet.

Salmon carcass meals

Two salmon carcass meals were fed in separate diets. Diet 2 contained the less-rancid meal prepared in 1961. This meal was manufactured by a dry-rendering method, and the solubles were not returned to the meal. The rancid salmon meal used in diet 3 was a precooked, pressed meal in which the solubles were defatted, dried, and returned to the meal. It was assumed that the 1963 meal did not receive adequate care after preparation by the manufacturer, which resulted in high rancidity.

Both the protein deposition and the average weight of fish fed the 1961 salmon meal were significantly greater at 12 and 24 weeks than the fish fed the 1963 salmon meal. These differences were evident after 4 weeks of feeding and progressively increased with time. Protein utilization was significantly better in fish fed the 1961 meal than in fish fed the 1963 meal, for both the 12- and 24-week periods. Fish fed the 1961 meal were larger than the fish fed the standard meat control diet, while fish fed the 1963 meal were smaller. Other deleterious effects of the 1963 salmon meal are described in the histological section of this report.

Herring meal

The protein deposition of fish fed diet 4 containing herring meal was inferior to that of fish fed the control diet (number 2) containing salmon meal. The average weight of fish fed diet 4 was less than that of fish fed diet 2, while fat deposition was greater in the diet 4 fish than in the control fish. Both the high fat deposition and the low protein deposition indicated that herring meal was a poor substitute for salmon meal under the conditions of this experiment.

Turbot meal

Turbot meal, fed in diet 5 was found to be an excellent growth producer. Deposition and utilization of protein by fish fed this meal as well as average weight and fat deposition were equal to those of fish fed the salmon meal.

Dogfish meals

Two lots of dogfish meal, obtained from different manufacturers and designated dogfish meal A and dogfish meal B, were used in diets 6 and 7. Protein deposition and utilization by fish fed dogfish meal A, diet 6, were significantly better than by fish fed dogfish meal B, diet 7. A difference in growth was evident after 2 weeks of feeding and progressively increased with time. The growth capability of dogfish meal A was equal to that of the salmon and turbot meals previously described, while that of dogfish meal B was significantly less than that of the salmon

Table 2:-- Chemical analyses ofingredients fed in the 1964 feeding trials

Ingredient	Proximate analysis (percent) 1/					Remarks
	Water	Protein	Fat	Ash	Carbohydrate (by difference)	
1961 Chinook salmon carcass meal	7.62	68.00	16.89	11.26	--	Prepared from spawned-out carcasses. This meal has been kept under refrigeration since its preparation in 1961.
1963 Chinook salmon carcass meal	6.21	72.11	10.89	11.09	--	in 1961. Prepared from spawned out carcasses.
1963 Herring meal	7.05	73.58	9.78	10.96	--	Prepared from whole carcasses.
1963 Turbot meal	7.24	60.46	12.82	18.72	--	Prepared from whole carcasses.
1963 Dogfish meal A	7.06	59.70	17.76	14.96	--	Prepared from whole carcasses.
1963 Dogfish meal B	5.35	67.36	13.19	14.31	--	Prepared from whole carcasses, but by a different manufacturer than the Dogfish meal A.
1963 Rockfish meal	7.33	49.68	13.84	26.06	--	Prepared from fillet scraps.
1963 Sole meal	3.32	51.35	14.94	31.07	--	Prepared from fillet scraps.
1963 tuna meal	3.10	64.28	17.44	14.40	--	Prepared from fillet scraps.
Dried skim milk	6.58	36.09	0.67	7.47	49.19	Spray process
Cottonseed meal	14.84	43.81	3.81	5.58	31.96	"Guaranteed" 44.0 percent protein.
Wheat germ meal	13.76	30.69	15.85	4.16	35.54	
Hog liver	68.90	18.40	5.90	1.30	5.60	
Beef spleen	78.00	15.80	4.80	1.40	--	
Distiller's solubles	12.40	30.70	12.70	8.80	40.50	

1/ Determined by Joseph W. Elliott at the Salmon-Cultural Laboratory.

and turbot meals and less than that of the meat control diet. The fat deposition of fish fed the diet containing dogfish meal B was significantly higher than that of fish fed the diet containing salmon meal. These high fat deposits, along with the poor protein utilization by these fish, indicated that dogfish meal B was of poor protein quality.

Differences between these two meals were explored further. Chemical analysis revealed that the dogfish meal producing the poorer growth rate contained 1.13 percent urea while the other dogfish meal contained 0.5 percent urea. The urea content of the dogfish meals would be included in the nitrogen analysis and therefore calculated as protein. As the diets were fed on a 25-percent protein basis, the meal containing the higher urea level would be fed at a proportionally lower protein level than the meal containing the lower urea content, and both meals would be fed below the true protein level. The calculated difference in protein content between the meals including the urea was 7.7 percent. With the urea excluded, the difference was 5.9 percent or a 1.8 percent difference due to urea. This difference did not explain the growth differential between the fish fed the two meals. Dogfish meal contains other nitrogenous products such as trimethylamine which, if present in significant amounts, could further reduce the calculated protein content.

As a separate experiment, a new group of fish was selected and fed to determine whether the growth differential was due to this calculated difference in protein content or to damage of protein quality in manufacturing or possibly to the adverse effect of urea. New diets were formulated so that each lot of fish received an equal quantity of dogfish meal in its ration disregarding the calculated protein contents of the meals. After 4 weeks of feeding, the group of fish receiving the dogfish meal low in urea, as in the previous experiment, had a superior growth rate indicating that this meal was of better quality than the dogfish meal high in urea or possibly that the fish were unable to utilize a fish meal this high in urea content.

Rockfish, sole, and tuna meals

Rockfish, sole, and tuna meals used in this experiment were fillet-scrap meals and were not prepared from the whole carcass as were the previously described meals. Rockfish proved best of the fillet-scrap meals. Fish from diet 8, which contained rockfish meal, had better growth rates than did fish receiving sole and tuna meals. None of the 3 fillet-scrap meal diets produced

growth or protein utilization comparable to that produced by the control diet containing the salmon carcass meal.

Combination fish meal diets

Diets 11 through 21 contained salmon, herring, or turbot meal in combination with the remaining meals tested and in combination with each other. These diets were formulated so that an equal amount of protein was supplied by each fish meal.

Several diets containing two fish meals produced protein deposition and utilization and average weight equal to those produced by the control diet containing salmon carcass meal. In no instance did the growth rates of the fish fed the diets containing two fish meals exceed those of fish fed the control salmon meal diet. Diets 13, 18, and 21 each contained the dogfish meal high in urea content described previously in this report. Each of these three diets produced fish with growth rates significantly less than those of fish fed diet 2. This reduction further indicated the poor quality of this dogfish meal. Two other diets which contained tuna meal, diets 16 and 20, produced fish with poor growth rates.

Histopathological examination

At the conclusion of the feeding trials, samples of fish were selected from diets 1 through 10 and diets 12 and 17 for histopathological examination. Diets 1 through 10 represented the control meat diet and each of the fish meals fed separately. Fish fed diet 12 were examined to determine whether the early feeding of the rancid salmon meal had any effect. Fish from diet 17 were selected to compare the effects of feeding a combination of fish meals with those of feeding a single fish meal.

Control meat diet.--Histopathological examination of preserved livers from fish fed the all-meat control diet revealed intracellular deposition of lipoproteins. This condition has been reported by Fowler and Wood (1966) and is believed to be related to hard fat in the diet. Further experiment has been reported by Elliott, Fowler, and Burrows (1966) in which chinook salmon fingerlings fed meat diets were characterized by poor growth, increased cholesterol levels, and decreasing hematocrits and lower corpuscular counts.

Meal diets.--Fish fed the meal diets, with the exception of diet 3, showed no abnormal histopathological conditions. All groups of fish had excellent relative distribution of liver and visceral fat, i. e. little or no lipid was in the liver tissue and visceral lipid stores were good. The group of fish fed diet 3, which contained the highly rancid salmon carcass meal, showed definite hepatotoxic changes. These changes were characterized by a diffuse loss of polarity of the cells in the hepatic plates, irregular staining, occasional pyknotic nucleii, and low fat and glycogen content. No other organic changes were seen.

The histological indications are that the decreased growth rate of fish fed diet 1 containing meat was associated with the hard fat content of this diet. The decreased growth rate of fish fed the highly rancid salmon meal was associated with hepatotoxic lesions. The variations in growth rate recorded with all other diets were not ascribed to disease, parasites, or toxicity, but were probably a direct reflection of the nutritional quality of these diets.

The results of the 1964 trials indicate that turbot and dogfish meals are adequate substitutes for salmon meal in the composite meal mixture. The method of preparation of the fish meal, as well as the source of the meal, affects the protein quality of the meal.

RESULTS OF THE 1965 FEEDING TRIALS

The 1965 feeding trials were a continuation of the substitution tests conducted in 1964 plus some alterations in diet composition and methods of handling to determine optimum levels of ingredients and limitations of procedures. These feeding trials consisted of 21 diets designed to measure the contribution of choline chloride,

Table 3.--Summary of 1964 feeding trials with chinook salmon, 12- and 24-week periods

Initial number per tank: 264 fish
Initial weight per tank: 500 grams
Initial average weight per fish: 1.89 grams
Initial number per pound: 240 fish

Period: 1/29/64 -- 7/14/64
Average water temperature: 53° F.
Diet Protein: Diet 1: 20.7%, Diets 2-21: 25%
Diet Calories: Diet 1: 1,432 cal./kg., Diets 2-21: 2,350 cal./kg.

	Composition		Average weight per fish (grams)		Mortality (percent)		Food conversion (wet)		Protein deposition (grams)		Protein utilization factor		Fat deposition (grams)	
	Ingredient	Percent of diet	12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.
Diet 1	Hog liver	60.00	10.5	24.6	0.19	0.38	2.14	2.43	386.0	957.6	2.60	3.05	206.4	247.8
	Beef spleen	30.00												
	1961 Salmon meal	5.00												
	Distiller's solubles	5.00												
Diet 2	1961 Salmon meal	18.24	11.4	30.4	0.10	0.38	1.93	2.05	411.0	1,198.3	2.96	3.14	274.6	439.1
	Meal mix 1/	13.86												
	Water	39.64												
	Peanut oil	8.26												
Diet 3	1963 Salmon meal	17.20	8.4	22.9	0.19	0.28	2.61	2.36	280.9	829.7	3.95	3.83	204.4	418.0
	Meal mix	33.86												
	Water	39.60												
	Peanut oil	9.34												
Diet 4	Herring meal	16.85	10.6	27.4	0.28	0.38	2.06	2.17	364.7	1,020.1	3.24	3.49	267.2	540.0
	Meal mix	33.86												
	Water	39.76												
	Peanut oil	9.53												
Diet 5	Turbot meal	20.51	11.5	30.7	0.10	0.10	1.95	2.04	404.6	1,227.6	3.05	3.10	306.4	489.6
	Meal mix	33.86												
	Water	36.97												
	Peanut oil	8.66												
Diet 6	Dogfish meal A	20.77	11.8	32.2	0.19	0.19	1.91	1.97	411.0	1,277.7	3.02	2.99	328.1	593.0
	Meal mix	33.86												
	Water	37.65												
	Peanut oil	7.72												
Diet 7	Dogfish meal B	18.41	8.8	21.6	0.19	0.28	2.48	2.52	269.9	731.4	4.20	4.35	274.8	620.0
	Meal mix	33.86												
	Water	38.89												
	Peanut oil	8.84												
Diet 8	Rockfish meal	24.15	10.4	27.5	0.28	0.28	2.12	2.17	359.4	1,074.0	3.31	3.32	283.6	552.0
	Meal mix	33.86												
	Water	34.20												
	Peanut oil	7.79												
Diet 9	Sole meal	24.96	9.6	24.2	0.10	0.28	2.24	2.36	315.9	887.2	3.62	3.80	260.9	487.1
	Meal mix	33.86												
	Water	33.25												
	Peanut oil	7.93												
Diet 10	Tuna meal	19.29	9.7	25.4	0.19	0.38	2.22	2.27	331.8	951.4	3.45	3.59	251.4	518.0
	Meal mix	33.86												
	Water	38.84												
	Peanut oil	8.01												

Diet 11	1961 Salmon meal Herring meal Meal mix Water Peanut oil	9.12 8.43 33.86 38.30 8.47	10.7	29.0	0.10	0.38	2.05	2.10	383.4	1,159.1	3.10	3.16	277.4	436.6
Diet 12	1961 Salmon meal Turbot meal Meal mix Water Peanut oil	9.12 10.25 33.86 38.30 8.47	10.5	29.1	0.10	0.19	2.08	2.05	385.2	1,160.6	3.05	3.09	292.9	579.2
Diet 13	1961 Salmon meal Dogfish meal B Meal mix Water Peanut oil	9.12 9.20 33.86 39.26 8.56	10.0	27.3	0.10	0.28	2.18	2.13	354.8	1,045.2	3.00	3.33	304.1	641.9
Diet 14	1961 Salmon meal Rockfish meal Meal mix Water Peanut oil	9.12 12.07 33.86 36.92 8.03	11.0	30.0	0.19	0.66	2.00	2.06	408.4	1,200.8	2.95	3.08	289.2	523.7
Diet 15	1961 Salmon meal Sole meal Meal mix Water Peanut oil	9.12 12.48 33.86 36.45 8.09	10.7	28.5	0.19	0.19	2.04	2.13	397.0	1,101.0	2.98	3.31	294.4	617.0
Diet 16	1961 Salmon meal Tuna meal Meal mix Water Peanut oil	9.12 9.64 33.86 39.24 8.14	9.7	26.3	0.10	0.19	2.28	2.21	350.6	1,036.6	3.32	3.33	276.6	446.9
Diet 17	Herring meal Turbot meal Meal mix Water Peanut oil	8.43 10.25 33.86 38.35 9.11	11.0	29.6	0.00	0.19	2.00	2.08	416.0	1,134.0	2.90	3.28	295.4	487.9
Diet 18	Herring meal Dogfish meal B Meal mix Water Peanut oil	8.43 9.20 33.86 39.31 9.20	10.6	27.6	0.00	0.00	2.12	2.18	384.4	1,029.1	3.14	3.52	292.6	625.8
Diet 19	Herring meal Rockfish meal Meal mix Water Peanut oil	8.43 12.07 33.86 36.97 8.67	10.9	28.2	0.10	0.28	2.04	2.15	410.0	1,124.6	2.94	3.24	288.0	448.6
Diet 20	Herring meal Tuna meal Meal mix Water Peanut oil	8.43 9.64 33.86 39.29 8.78	10.2	27.8	0.10	0.10	2.16	2.16	378.0	1,087.6	3.16	3.32	259.4	487.4
Diet 21	Dogfish meal B Turbot meal Meal mix Water Peanut oil	9.20 10.25 33.86 37.93 8.76	10.2	27.1	0.00	0.38	2.14	2.17	367.6	1,025.7	3.21	3.42	318.8	538.9
Least difference at the 5% confidence level:			1.3	2.6	0.46	0.74			53.6	111.0	0.50	0.39	61.5	99.0

1/ Meal mix consists of 46.16 percent dried skim milk, 30.77 percent cottonseed meal, and 23.07 percent wheat germ meal.

274-595 O - 67 - 2

vegetable meals, dreid buttermilk, dried skim milk, and wheat germ as substitutes for or adjuncts to the basal ration. The length of time the diet could be prepared in advance of feeding and the necessity of keeping the basal meal under refrigeration were also tested.

The standard meat diet was no longer used as a control. Instead, the all-meal diet described in the section on the 1964 feeding trials, using the 1961 salmon carcass meal, was substituted. A second diet, using a 1964 salmon carcass meal, was used as a second control since the remaining diets were formulated with this meal. No difference could be determined due to growth, mortality, or hematological characteristics between fish fed diets containing either the 1961 or 1964 salmon meals. The chemical analyses of these meals and the other meals used in these experiments are presented in table 4.

All 21 diets were carried through to completion of the 24-week feeding trial, even though the fish fed some of the diets were experiencing heavy losses. A complete summary as well as the diet variables of this experiment are presented in table 5.

Effect of supplemental choline chloride

Choline chloride is one of the vitamins defined as necessary for chinook salmon (Halver, 1957). Considerable amounts of choline are in the Abernathy diet ingredients; the need for additional crystalline choline in the vitamin package was tested by feeding one group of fish a diet supplemented with choline, diet 3, and comparing them with fish fed the unsupplemented control, diet 2.

The additional choline did not appear to be of measurable benefit. No differences could be shown between fish fed these two diets as measured by total weight, growth, mortality, hematology, or histological examination. Of particular interest was the histological examination of fat deposits in the livers. Fish from the two diets had similar liver deposits, indicating that the lipotropic factors in the control diet were adequate without the additional choline. Previous experiments (Fowler et al., 1964) had indicated that choline when mixed with other vitamins may have caused a deleterious effect on the vitamin mixture. This effect was circumvented by adding the choline as a separate entity. The complete elimination of choline makes the diet simpler and more economical.

Various vegetable oils as caloric sources

Peanut oil has been used successfully as the caloric source in our diet experiments for several years. The disadvantage of peanut oil is that it is expensive. Corn, cottonseed, safflower, and soybean oils were tested as isocaloric replacements for peanut oil. These oils were used in diets 7 through 10, with diet 2 (peanut oil) serving as the control.

The one oil which proved superior was soybean. This oil when fed in diet 10 produced fish with significantly higher levels of protein deposition and total weight. The other oils were not different from the peanut oil. The substitution of soybean oil for peanut oil is of sizable benefit in that it is more economical and produces more growth.

Protein quality of several vegetable meals

In searching for means of improving and increasing the flexibility of the Abernathy diet, dehulled cottonseed, corn gluten, safflower, and soybean meals were tested as replacements for the regular cottonseed meal used in the control diet. These meals were tested on an isoprotein basis to determine the quality of the protein.

Dehulled cottonseed meal.--Dehulled cottonseed meal, 49.95 percent protein, did not prove different from the regular cottonseed meal, 43.81 percent protein, when fed on an isoprotein basis. Total weight, protein deposition and utilization, mortality, and hematological characteristics were not different between fish fed diets 2 and 11. The cost per unit of protein for the dehulled meal was less than for the regular meal, but the amount of vegetable oil added to these diets to make them isocaloric was higher

Table 4.—Chemical analyses of ingredients fed in the 1965 feeding trials.

Ingredient	Proximate analysis (percent)1/					Remarks
	Water	Protein	Fat	Ash	Carbohydrate (by difference)	
1961 Chinook salmon carcass meal	7.62	68.00	16.89	11.26	—	Prepared from spawned-out carcasses. This meal has been kept under refrigeration since its preparation in 1961.
1964 Chinook salmon carcass meal	7.10	75.98	11.50	7.8	—	Prepared in 1964 from spawned-out carcasses.
Dried skim milk	6.58	36.09	0.67	7.47	49.19	Spray process
Cottonseed meal	14.84	43.81	3.81	5.58	31.96	"Guaranteed" 44.0 percent protein
Wheat germ meal	13.76	30.69	15.85	4.16	35.54	
Dehulled cottonseed meal	10.00	49.95	1.96	6.53	31.56	Solvent extracted "guaranteed" 50.0 percent protein
Corn gluten meal	10.11	41.31	3.58	2.78	42.22	
Safflower meal	9.32	40.94	2.62	7.76	39.36	Solvent extracted
Soybean meal	9.00	51.13	1.85	5.74	32.28	Solvent extracted
Dried buttermilk	4.10	30.34	5.00	8.22	52.34	Roller process

1/ Determined by Joseph W. Elliott at the Salmon-Cultural Laboratory

in the dehulled meal diet because of its lower fat content. This oil differential made the cost of the two diets comparable.

One advantage of the dehulled meal was the absence of these hulls in the excrement of the fish, which meant less of a cleaning problem and would be of value on a production basis.

Corn gluten meal.--Corn gluten meal proved to have two disadvantages when compared with cottonseed meal. Mortality was significantly higher among fish fed diet 12 containing corn gluten meal than among fish fed diet 2, 7.33 percent against 0.56 percent. The mean total corpuscular count of 1,117,000 cells/cu. mm. for fish on the control diet. In this experiment corn gluten meal was an unsatisfactory substitute for cottonseed meal.

Safflower meal.--Fish fed diet 13 containing safflower meal had significantly higher mortality than fish fed the control diet. The high death rate was first evident after 18 weeks of feeding. From then to the end of the feeding trials, mortality increased markedly. The rate of gain of these fish also started to decline, and although the rate of gain was not significantly less than that of the control fish, the difference was approaching significance. The high mortality precluded the use of safflower meal as a replacement for cottonseed meal.

Soybean meal.--Soybean meal has been tested at our laboratory previously as a replacement for cottonseed meal (Fowler et al., 1966). It was unsuccessful as a replacement, but part of the problem was felt to be the palatability of the meal which resulted in a reluctance on the part of the fish to accept the diet during the first few weeks of feeding. The soybean meal tested in the 1965 trials was of a higher protein content than that used previously. On an isoprotein basis, less soybean meal would be included in a diet using this higher protein meal, which would tend to increase the palatability.

The fish readily accepted diet 14 containing soybean meal from initial feeding. At the end of 24 weeks, mortality was higher, total weight less, protein deposition less, and protein utilization less efficient than that of fish fed the control diet. These deficiencies made soybean meal a poor choice as a substitute for cottonseed meal.

Table 5.—Summary of 1965 feeding trials with chinook salmon, 12- and 24-week periods

Initial number per tank: 539 fish
Initial weight per tank: 500 grams
Initial average weight per fish: 0.93 grams
Initial number per pound: 488 fish

Period: 1/21/65 — 7/7/65
Average water temperature: 53° F.
Diet Protein: 25%
Diet Calories: 2,350 cal./kg.

	Composition[1]		Average weight per fish (grams)		Mortality (percent)		Food conversion (wet)		Protein deposition (grams)		Protein utilization factor		Fat deposition (grams)		Percent hematocrit	Total blood cell counts cells/cu. mm.	Performance rating	Mean total weight (grams)	
	Ingredient	Percent of diet	12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.	24 wks.	24 wks.	24 wks.	12 wks.	24 wks.
Diet 1	1961 Salmon meal Dried skim milk Cottonseed meal Wheat germ Peanut oil	18.24 15.63 10.42 7.81 8.54	6.2	18.0	0.37	1.67	1.78	1.83	446.0	1,432.6	2.82	2.86	290.2	602.7	28.9	1,274,000	—	3,328	9,452
Diet 2	1964 Salmon meal Dried skim milk Cottonseed meal Wheat germ Peanut oil	16.32 15.63 10.42 7.81 9.61	5.8	18.2	0.18	0.56	1.87	1.78	416.0	1,436.0	2.96	2.82	266.4	626.8	32.2	1,455,000	93.8	3,138	9,580
Diet 3	1964 Salmon meal Dried skim milk Cottonseed meal[2] Wheat germ Peanut oil	16.32 15.63 10.42 7.81 9.61	6.2	18.2	0.00	0.46	1.80	1.80	439.0	1,482.7	2.89	2.80	312.6	547.2	33.2	1,511,000	—	3,306	9,712
Diet 4	1964 Salmon meal Dried skim milk Cottonseed meal[3] Wheat germ Peanut oil	16.32 15.63 10.42 7.81 9.61	6.0	18.2	0.09	1.20	1.84	1.82	434.5	1,460.7	2.91	2.81	250.8	599.0	28.6	1,341,000	—	3,249	9,556
Diet 5	1964 Salmon meal Dried skim milk Cottonseed meal[4] Wheat germ Peanut oil	16.32 15.63 10.42 7.81 9.61	5.9	17.5	0.46	1.76	1.87	1.88	408.6	1,402.4	3.06	2.90	263.7	450.9	30.0	1,390,000	51.6	3,176	9,156
Diet 6	1964 Salmon meal Dried skim milk Cottonseed meal[5] Wheat germ Peanut oil	16.32 15.63 10.42 7.81 9.61	6.2	17.2	0.09	2.50	1.83	1.95	427.4	1,401.8	3.01	2.95	265.0	461.7	28.8	1,250,000	10.7	3,314	8,974
Diet 7	1964 Salmon meal Dried skim milk Cottonseed meal Wheat germ Corn oil	16.32 15.63 10.42 7.81 9.61	6.0	17.9	0.46	1.02	1.87	1.80	414.7	1,444.4	3.02	2.80	265.4	619.4	33.4	1,435,000	—	3,178	9,474
Diet 8	1964 Salmon meal Dried skim milk Cottonseed meal Wheat germ Cottonseed oil	16.32 15.63 10.42 7.81 9.61	6.0	18.0	0.18	0.56	1.85	1.80	415.1	1,444.2	3.02	2.82	283.0	691.4	34.5	1,578,000	—	3,203	9,550
Diet 9	1964 Salmon meal Dried skim milk Cottonseed meal Wheat germ Safflower oil	16.32 15.63 10.42 7.81 9.61	6.0	18.2	0.46	1.02	1.84	1.79	421.2	1,435.4	2.98	2.83	288.0	625.8	33.6	1,405,000	—	3,226	9,576
Diet 10	1964 Salmon meal Dried skim milk Cottonseed meal Wheat germ Soybean oil	16.32 15.63 10.42 7.81 9.61	6.2	19.0	0.09	0.37	1.79	1.74	444.4	1,532.1	2.86	2.74	310.4	694.7	30.2	1,417,000	113.2	3,323	10,112

Diet 11	1964 Salmon meal 16.32 Dried skim milk 15.63 Cottonseed meal(delnulled) 9.13 Wheat germ 7.81 Peanut oil 9.88	6.4	18.4	0.28	1.76	1.76	1.83	454.4	1,456.2	2.84	2.88	318.5	599.8	32.0	1,413,000	89.2	3,422	9,642
Diet 12	1964 Salmon meal 16.32 Dried skim milk 15.63 Corn gluten meal 11.04 Wheat germ 7.81 Peanut oil 9.37	6.4	18.8	0.46	7.33	1.76	1.90	458.2	1,432.3	2.80	2.90	279.0	613.4	25.2	1,117,000	—	3,410	9,252
Diet 13	1964 Salmon meal 16.32 Dried skim milk 15.63 Safflower meal 11.14 Wheat germ 7.81 Peanut oil 9.52	6.5	19.2	0.56	10.02	1.74	1.92	466.6	1,412.6	2.80	2.95	317.8	622.0	28.7	1,226,000	—	3,488	9,170
Diet 14	1964 Salmon meal 16.32 Dried skim milk 15.63 Soybean meal 8.92 Wheat germ 7.81 Peanut oil 9.90	5.9	17.4	0.56	7.88	1.86	1.98	415.0	1,318.6	2.99	3.02	260.4	683.4	28.2	1,269,000	—	3,157	8,544
Diet 15	1964 Salmon meal 16.32 Dried buttermilk 18.59 Cottonseed meal 10.42 Wheat germ 7.81 Peanut oil 8.51	6.3	18.5	0.09	1.02	1.78	1.81	451.5	1,529.2	2.85	2.74	324.0	745.2	30.8	1,353,000	—	3,390	9,763
Diet 16	1964 Salmon meal 23.74 Cottonseed meal 10.42 Wheat germ 7.81 Peanut oil 10.31	5.6	13.0	0.28	24.49	1.94	3.14	392.6	869.8	3.10	4.18	255.0	267.0	22.6	980,000	7.9	3,006	5,134
Diet 17	1964 Salmon meal 21.89 Dried skim milk 3.91 Cottonseed meal 10.42 Wheat germ 7.81 Peanut oil 10.12	5.9	16.0	0.28	10.95	1.86	2.27	421.7	1,148.3	2.97	3.44	262.8	489.6	22.9	1,165,000	—	3,189	7,439
Diet 18	1964 Salmon meal 20.03 Dried skim milk 7.82 Cottonseed meal 10.42 Wheat germ 7.81 Peanut oil 9.96	6.2	17.4	0.18	5.75	1.79	1.99	438.2	1,344.8	2.87	3.01	304.2	549.3	29.3	1,315,000	—	3,308	8,647
Diet 19	1964 Salmon meal 18.18 Dried skim milk 11.72 Cottonseed meal 10.42 Wheat germ 7.81 Peanut oil 9.78	6.0	17.6	0.09	1.67	1.82	1.87	421.7	1,386.8	2.99	2.93	286.7	623.5	31.2	1,393,000	—	3,275	9,174
Diet 20	1964 Salmon meal 17.46 Dried skim milk 16.74 Cottonseed meal 11.16 Wheat germ 2.60 Peanut oil 10.38	6.1	17.6	0.09	0.37	1.85	1.87	427.3	1,400.6	2.98	2.94	284.5	533.0	27.0	1,278,000	—	3,262	9,312
Diet 21	1964 Salmon meal 16.89 Dried skim milk 16.18 Cottonseed meal 10.79 Wheat germ 5.21 Peanut oil 10.00	6.5	18.8	0.09	0.46	1.76	1.80	462.0	1,504.6	2.84	2.82	278.0	686.0	29.6	1,390,000	109.0	3,492	9,930
Least difference at the 5% confidence level:		0.6	0.8	0.57	5.44			53.6	82.2	0.36	0.17	88.7	174.4	7.5	278,000	34.8	332	514

1/ Unlisted ingredients consist of 2 percent vitamin-CMC mixture and remainder of water.
2/ Choline chloride added at the rate of 60.00 mg. per kilogram of fish per day.
3/ Diet prepared every third day.
4/ Diet prepared every seventh day.
5/ Dry meals premixed and held at room temperature for the entire trial period.

Dried buttermilk as a substitute for dried skim milk

Dried buttermilk was tested as a replacement for dried skim milk on an isoprotein basis in diet 15. The results indicated that buttermilk was comparable in quality to dried skim milk, if not better. Protein deposition of the fish fed the diet containing buttermilk was significantly greater than that of fish fed diet 2 containing dried skim milk. This increase was not measurable in the total weight or average weight of these two lots of fish, indicating that the difference in protein deposition may not have been valid. Retesting of the buttermilk is needed before an accurate evaluation can be made.

Effect of the amount of dried skim milk in the basal ration

One of the more interesting segments of this experiment was the series of diets which tested the amount of dried skim milk necessary in the basal ration. Diet 2, the control, contained 15.63 percent dried skim milk. In diets 16 through 19 the amounts of dried skim milk were 0.00 percent, 3.91 percent, 7.82 percent, and 11.72 percent; the increments were 25 percent of the amount in the control diet. The diets were maintained on an isoprotein basis by replacing the dried skim milk with salmon carcass meal.

After the first weigh period, fish fed diet 16 containing no dried skim milk showed a reduced growth rate. This retardation continued as the experiment progressed. Mortality for this group of fish started to increase at the end of 14 weeks. At the end of 24 weeks, definite trends could be seen in the entire group of diets which were correlated with the amounts of dried skim milk fed. In fish fed diets 16 through 19 and the control diet 2, total weight, protein deposition, protein utilization, and fat deposition increased as the dried skim milk in the diet was increased. Conversely, mortality of these fish decreased as the dried skim milk increased. Total blood cell counts of the fish fed these diets also tended to increase as the dried skim milk increased. These results are presented in table 6.

Diet 19 did not differ significantly from the control diet 2. The trends noted, however, indicated that diet 19 might be a marginal diet nutritionally and would not be safe in production operations. The general condition and appearance of the fish fed diets 16, 17, 18, and 19 were not as good as those fed the control diet. Fish receiving the decreased amounts of dried skim milk were characterized by dark coloration, anorexia, loss of equilibrium, and inability to maintain themselves in a current. These deficiencies were, again, correlated with the amounts of dried skim milk fed.

When the increase in mortality of fish fed diet 16 was noted, we first interpreted it as a possible vitamin deficiency. Since choline chloride was the only vitamin not in the vitamin package, the diet in one of the paired tanks was altered by the addition of choline from the beginning of the 16th week until the end of the feeding trials. The remaining tank of fish was maintained on the original diet. The addition of choline chloride did not improve the condition of these fish or lower the mortality. We now feel that the nutritional deficiency evident in these diets might have been caused by an amino acid imbalance.

Effect of the amount of wheat germ in the basal ration

Wheat germ was fed in the control diet, diet 2, at a rate of 7.81 percent of the diet. It was hypothesized that perhaps the protein quality of the wheat germ was not as good as that of the protein furnished by the other components of the basal ration. Reducing the amount of wheat germ fed and replacing it on an isoprotein basis by salmon carcass meal, dried skim milk, and cottonseed meal, might increase the growth of the fish over that of the control diet. Diets 20 and 21 were formualted with 2.60 and 5.21 percent wheat germ respectively, which amounted to one-third and two-thirds of that contained in the control diet.

Table 6.—Effect of increases of dried skim milk

Diet	Percent dried skim milk in the diet	Mortality (Percent) 24 weeks	Total weight (grams) 24 weeks	Protein deposition (grams) 24 weeks	Protein utilization factor 24 weeks	Fat deposition (grams) 24 weeks	Total blood cell counts - cells/cu. mm. 24 weeks
16	0.00	24.49	5,134	869.8	4.18	267.0	980,000
17	3.91	10.95	7,439	1,148.3	3.44	489.6	1,165,000
18	7.82	5.75	8,647	1,344.8	3.01	549.3	1,315,000
19	11.72	1.67	9,174	1,386.8	2.93	623.5	1,393,000
2	15.63	0.56	9,580	1,436.0	2.82	626.8	1,455,000

At the end of 24 weeks of feeding, no differences between fish fed the control diet and fish fed the diets with decreasing amounts of wheat germ were shown by total weight, mortality, protein deposition and utilization, or hematological characteristics. These results have been tabulated in table 7. There was a difference in total weight and protein deposition of fish fed diets 20 and 21; diet 21 fish were significantly larger. This difference may have indicated that the low percentage of wheat germ fed in diet 20 was inadequate and that the protein quality of wheat germ was better than we had assumed.

A trend toward an increase in the total blood counts and hematocrits as the amount of wheat germ in the diet increased was indicated at the conclusion of the experiment. Fish fed diet 20, lowest in wheat germ, had the lowest hematological measurements, while fish fed the control diet had the highest. This correlation did not prove statistically valid but was approaching validity ($r = 0.802$; $P\ 0.05 = 0.811$). Increased replication may have made these data significant.

The wheat germ in the diet is the chief supplier of vitamin E. Woodall et al. (1964) found that diets deficient in tocopherol, when fed to chinook fingerlings, caused poor growth, exophthalmia, ascites, erythrocyte fragility, anemia, clubbed gills, epicarditis, and ceroid deposition in the spleen. More recently, Whitmore (1965) described a microcytic anemia in juvenile chinook salmon fed diets deficient in vitamin E. Both reports indicate that salmon fingerlings require vitamin E, and since wheat germ meal is an excellent cheap source of this vitamin and since there is an indication of improved hematology at the highest level fed, no alteration in the present level of wheat germ meal in indicated.

Effect of storage on prepared diets

The control diet, diet 2, was prepared each day and fed the same day. On a production basis considerable time would be saved if several days' feed could be prepared at one time and stored under refrigeration until fed. To test the effect on fish, diets were prepared several days in advance. Diet 4 was prepared in sufficient quantity for 3 days of feeding and diet 5 for 7 days. Both diets were stored under refrigeration until consumed.

No differences could be measured between fish fed the diet prepared fresh daily, and fish fed the diet prepared every 3 days. Storage presented no feeding problems as this diet fed as readily through the ricer after 3 days of storage as it did when first prepared. On a production basis, considerable time in preparing the diet and cleaning equipment would be saved by this method.

Fish fed diet 5, which was prepared for a 7-day period, showed a reduction in the rate of gain during the latter weeks of the experiment. Analysis of the fat deposition of these fish indicated the level to be significantly lower than that of the control fish. In addition to the reduction in fat depots, the performance of the fish fed diet 5 as measured by the stamina tunnel was inferior to that of the control fish.

Table 7.—Effect of increases of wheat germ

Diet	Percent of wheat germ in the diet	Mortality (percent) 24 weeks	Total weight (grams) 24 weeks	Protein deposition (grams) 24 weeks	Protein utilization factor 24 weeks	Protein deposition (grams) 24 weeks	Total blood cell counts - cells/cu. mm. 24 weeks
20	2.60	0.37	9,312	1,400.6	2.94	533.0	1,278,000
21	5.21	0.46	9,930	1,504.6	2.82	686.0	1,390,000
2	7.81	0.56	9,580	1,436.0	2.82	626.8	1,455,000

Effect of storing the basal meal mixture at room temperature

The normal procedure at this laboratory is to keep all meals used in diet formulation under refrigeration at minus 10° F. until diet preparation. A considerable saving could be realized if refrigeration was not necessary. Diet 6 was used to test the necessity of refrigeration by premixing the complete 24-week supply of dry meals and storing them at room temperature. The dry meal mixture for the control diet was premixed but stored under refrigeration.

The first indication that fish from diet 6 were being affected by this procedure was at the end of 20 weeks of feeding. Until then no differences between fish fed the two diets could be discerned. After this a few darkly pigmented fish were noticed and, as time progressed, these affected fish became more numerous until roughly 25 percent of the total number were dark. These abnormal fish also exhibited anorexia and a decreased ability to maintain themselves in the water current.

The mortality of the fish fed diet 6 was 1.95 percent at the end of the feeding trial. This rate is not significant when it is compared with that of fish fed the control diet, but interestingly enough, this mortality was all during the last 8 weeks of the experiment. Also, during the latter part of the experiment, the biweekly percent gains began to drop markedly. Although this reduction in rate of gain was not reflected in a difference in the protein deposition, it was shown in the mean total weight which was lower at the end of the feeding trials than that of the control fish. Stamina tests run of the diet 6 fish revealed them to be extremely poor swimmers. Their performance rating was 10.7, significantly lower than that of the control fish, which was 93.8.

Rancidity values for the meal used in diet 6 were determined monthly, since it was expected that this meal would tend to become rancid as time progressed. The meal proved stable; there was no difference between initial and final rancidity values. The effect this meal had on the fish must be attributed to unknown factors.

SUMMARY

1964 feeding trials

The results of the 1964 feeding trial may be summarized as follows:

Turbot meal and a dogfish meal, low in urea content, were found to be suitable substitutes for salmon carcass meal.

A rancid salmon carcass meal produced poor growth. Histological examination of livers from fish fed this meal showed definite evidence of hepatotoxicity.

Herring meal and a dogfish meal, high in urea content, as well as fillet-scrap meals, rockfish, tuna, and sole, produced inferior growth when compared to salmon carcass meal.

Fish meals fed in combinations did not produce better growth rates than diets containing a single fish meal.

Fish fed a meat control diet had less growth and developed lipoprotein deposits in the liver, both of which were assumed to be associated with the hard-fat content of the diet.

1965 feeding trials

The results of the 1965 feeding trial are summarized as follows:

A salmon carcass meal prepared in 1961 and held under refrigeration produced growth equal to that of a salmon meal prepared in 1964.

Supplemental crystalline choline chloride in the diet did not produce measurable benefits.

Soybean oil when used as a caloric source produced a significantly greater sparing action on the protein than did peanut oil, corn oil, cottonseed oil, or safflower oil.

A dehulled cottonseed meal had as good a protein quality as regular cottonseed meal. Corn gluten, safflower, and soybean meals when fed as substitute diet components caused higher mortality than diets containing the cottonseed meals.

Dried buttermilk, used as a replacement for dried skim milk, resulted in comparable growth rates.

Decreasing amounts of dried skim milk and increasing amounts of salmon meal in the diet were correlated with decreased fish growth and increased mortality. Total blood cell counts also decreased as the skim milk was decreased.

Decreasing the amount of wheat germ and increasing the combination of salmon meal, dried skim milk, and cottonseed meal proportionately resulted in a trend toward decreased total blood cell counts but did not affect growth or death rates of fish fed these combinations.

Preparing a diet in 7-day allotments and feeding it for 7 consecutive days resulted in fish with poor stamina and reduced fat stores. Diets prepared and fed in the same manner, but in 3-day allotments did not have these adverse effects.

A diet formulated from dry meals not kept under refrigeration produced fish with dark coloration, lowered total weight, and reduced swimming ability after 20 weeks of feeding.

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U. S. GOVERNMENT PRINTING OFFICE : 1967 O - 274-595

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