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FURTHER STUDIES OF PROTEIN AND CALORIE LEVELS OF MEAT-MEAL, VITAMIN-SUPPLEMENTED SALMON DIETS

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Salmon-Cultural Laboratory
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ABSTRACT

Feeding trials were conducted with fingerlings of fall chinook salmon (Oncorhynchus tshawytscha) to determine the effect of feeding three protein levels, four caloric levels, crystalline vitamins, and meat supplements in a basic meal mixture. After 24 weeks of feeding the results were as follows:

Fish fed at a protein intake of 27.5 percent produced significantly higher protein deposition than did fish fed at protein intakes of 25 and 20 percent, but tended to be less efficient as measured by protein utilization.

Addition of supplemental energy calories in the diets resulted in a sparing action on the protein and vitamin requirements of the fish. The energy requirement of the fish increased, in proportion to the protein requirement, as the fish increased in size.

A crystalline vitamin supplement was inadequate when fed in diets containing a meat supplement.

Meat supplementation ranging from 10 to 50 percent of the meat-meal combination produced no measurable differences in mortality, average weight, protein deposition, or protein utilization.

An all-meal diet produced fish with gains, protein deposition, protein utilization, performances, and general condition as good as or better than fish in comparable diets fed meat supplements.

Feeding trials conducted at the Salmon-Cultural Laboratory, Longview, Washington, in 1962, were a continuation and enlargement of experiments reported by Combs et al. (1962). Fingerlings of fall chinook salmon (Oncorhynchus tshawytscha) were used as the test animals. The primary purpose of these and the previous trials was the development of nutritionally adequate and economically feasible diets for the artificial propagation of salmon.

The experiments were directed toward exploring the effect of several protein levels, caloric intakes, and meat and vitamin supplementation when fed in a single basic meal ration. The composite-meal, basal ration was fed with a meat supplement ranging in amounts from 10 to 50 percent of the total mixture and with or without a complete vitamin supplement. The level of protein intake was controlled by partial reconstruction of the diet with water by the method developed by Phillips and Brockway (1959), and the level of calories was increased when desired by the addition of peanut oil. With this technique it was possible to determine the effect of energy calorie supplementation at several fixed levels of protein intake.

The 1962 feeding trials demonstrated the practicability of the meal combination, the effect of three protein levels on growth, and the effect of energy calorie supplementation on protein utilization. It was also demonstrated that an all-meal diet was as successful as comparable meat-supplemented diets and that vitamin supplementation could not be shown to be of any measurable value when fed in diets containing a meat supplement.

Roger E. Burrows, Bobby D. Combs, and Dr. Wilton W. Heinemann assisted in design of the experiment and interpretation of results; Joseph W. Elliott made the chemical analysis of the fish and diet components.

METHODS AND TECHNIQUES

The methods, techniques, and equipment used in the 1962 feeding trials were similar to those used in previous trials at this laboratory. Samples of fish weighing 500 grams were drawn from a homogeneous population and placed randomly in paired tanks. At bi-weekly intervals the fish in each tank were weighed and from these data the amount of food fed was calculated from feeding charts developed for chinook salmon. The food was ricer-fed with the frequency of feeding dependent on the time required for consumption.

The chemical composition of feeds was used as the basis for diet formulation, with caloric levels calculated on the basis of available calories as developed by Phillips and Brockway (1959). The chemical composition of the fish, as determined by proximate analysis, was the criterion used for analyzing the results of the experiments. Protein deposition was used as the measure of growth instead of total gain in weight. Protein utilization, a measure of the efficiency of a diet for growth production, was calculated by dividing the amount of protein fed by the amount of protein deposited in the fish. At the conclusion of the feeding trials, the data were analyzed statistically by use of analysis of variance for paired experiments as outlined by Snedecor (1956).

The techniques employed are similar to those used in previous feeding trials as described by Burrows et al. (1951; 1952) and by Combs et al. (1962) with the following exceptions:

1. The difficulties encountered in the 1961 experiments (Combs et al., 1962) were eliminated by the use of pathogen-free well water at a constant temperature of 53° F. and the housing of the experimental tanks within the laboratory to remove the possibility of diet supplementation with natural foods.

2. The original control diet was the Entiat production diet which had been fed on a production basis at Entiat, Washington, for several years and at Longview during 1961 with excellent results, but always on an increasing water temperature. This diet was marginal in vitamin content and when fed on a constant water temperature where the intake of the fish was much lower than at a rising temperature, a severe anemic condition became apparent at the end of eight weeks which resulted in the termination of this group of fish as a control. A new group of fish was substituted and placed on the Leavenworth production diet with satisfactory results. Table 1 gives the basic components of the diets and the vitamin supplement fed. The ingredients of the basic mixtures and their proximate analysis are shown in table 1A.

3. The feeding trials were carried on for a 24-week period. Data included in this report were compiled at the end of 12 weeks and at the end of 24 weeks.

4. Stamina tests were run on fish from each diet at the conclusion of the experiments, utilizing the stamina-tunnel device developed at this laboratory. Two 100-fish samples were tested from each diet and a performance index calculated.

5. Vitamin injection studies were conducted after the regular feeding experiments in an attempt to determine the cause of a nutritional deficiency which became apparent in all the experimental diets after 16 weeks of feeding. Affected fish from low-caloric, low-protein diets were selected to be used for the studies.

Table 1.--Basic components used in diets

A. Basic mixtures fed in the 1962 feeding trials

	Percentage Composition	Proximate analysis (percent)				
		Water	Protein	Fat	Ash	Nitrogen-Free Extract
Entiat Production Diet (Control I)	100.0	68.4	19.8	6.5	2.5	2.8
Hog liver	12.5	68.9	18.4	5.9	1.3	5.6
Beef spleen	12.5	78.0	15.8	4.8	1.4	
Arrowtoothed halibut	25.0	72.9	15.9	8.0	3.3	
Salmon viscera	40.0	77.3	16.6	4.5	1.2	0.4
1/ Salmon carcass meal	5.0	5.4	68.0	16.9	11.3	
Distiller's solubles	5.0	12.4	30.7	9.6	6.8	40.5
Leavenworth Production Diet (Control II)	100.0	67.0	20.8	6.4	2.1	3.7
Hog liver	20.0	68.9	18.4	5.9	1.3	5.6
Beef liver	20.0	67.6	20.4	6.9	1.3	3.8
Beef spleen	20.0	78.0	15.8	4.8	1.4	
Salmon viscera	30.0	77.3	16.6	4.5	1.2	0.4
Salmon carcass meal	5.0	5.4	68.0	16.9	11.3	
Distiller's solubles	5.0	12.4	30.7	12.7	6.8	40.5
A-1 Meal Mixture	100.0	8.4	48.0	9.2	7.9	26.5
Salmon carcass meal	35.0	5.4	68.0	16.9	11.3	
Dried skim milk	30.0	6.6	36.1	0.7	7.5	49.2
Cottonseed meal	20.0	14.8	43.8	3.8	5.6	32.0
Wheat germ	15.0	13.7	30.7	15.6	4.2	35.5
Meat Mixture	100.0	68.2	19.4	6.4	1.3	4.7
Beef liver	50.0	67.6	20.4	6.9	1.3	3.8
Hog liver	50.0	68.9	18.4	5.9	1.3	5.6

1/ Prepared from spawned-out carcasses of chinook salmon. The carcasses were steam cooked and semi-vacuum dried. The solubles were dried and returned to the meal.

The control diet was bound by the addition of two grams of salt per 100 grams of diet.

The experimental diets were bound by the addition of two grams of salt and two grams of CMC per 100 grams of diet.

B. Composition of vitamin supplement

Thiamine	.20 mg.	Inositol	20.00 mg.
Riboflavin	1.00	Biotin	.04
Pyridoxine	.43	Folic acid	.15
Choline	60.00	Total	90.82
Niacin	7.00	CMC	409.18
Pantothenic acid	2.00		500.00 mg.

Lots of 30 fish each were injected intraperitoneally with a 10-day supply of the B-complex vitamins. The injections were with single vitamins, groups of vitamins, and the entire B-complex with the exception of choline chloride.

RESULTS OF EXPERIMENTS

The test diets included a standard control and 20 experimental diets which were designed to measure the effect of four levels of meat supplementation, the addition of a crystalline vitamin supplement, three protein levels, and four caloric levels. A summary of the results of this experiment compiled at 12 weeks and 24 weeks is presented in table 2. At the conclusion of the feeding trials fish from each of the experimental diets were showing symptoms of hypovitaminosis. With the exception of the low-calorie, 20-percent protein diets, the remaining experimental diets produced gains comparable to or exceeding that produced by the control diet.

Effect of meat and vitamin supplementation

12 weeks.--Diets containing the highest level of meat supplementation tended to produce fish with greater protein deposition and better protein utilization at each of the three protein levels fed, but these differences were not significant. Meat supplementation did not have any effect upon mortalities. At the 20-percent protein level, diet 6, which was vitamin supplemented, had significantly less mortality than the comparable unsupplemented ration, diet 5. There was no difference in growth. No differences in either mortalities or growth could be shown in the 25-percent protein diets due to vitamin supplementation.

24 weeks.--In the low-calorie 20 and 25 percent protein diets, no difference due to the amount of meat supplementation could be seen in either mortalities, average weight, protein deposition, or protein utilization. In the high-calorie, 25-percent protein diets, fish from the higher meat-supplemented diets, diets 11 and 12, had significantly greater protein deposition than did fish from comparable diets containing the 10-90 combination, diets 16 and 17. The trend was for the fish from the higher meat-supplemented diets to have better protein utilization and to have fewer mortalities, but these differences were not significant. No differences due to meat supplementation were shown in the 27.5-percent protein diets but again the trend was for the fish in diet 19 witn 30 parts of meat to have greater protein deposition and better utilization than fish in diet 20 with 10 parts meat. Table 3 summarizes the results of the 25- and 27.5-percent protein diets at the end of 24 weeks.

The hypovitaminosis present at the conclusion of the experiments undoubtedly obscured the effect of the meat supplementation. It does appear that protein deposition was greater and protein utilization was better at the higher levels of meat supplementation when the caloric and protein levels were increased.

An increase in growth or a decrease in mortalities could not be demonstrated when a crystalline vitamin supplement was added to diets which included a meat supplement (table 4). Whether the vitamin supplement had any beneficial effect upon the all-meal diet has yet to be tested. Several possibilities as to why the crystalline vitamin supplement was not effective or available to the fish when fed in conjunction with the meat supplement can be postulated. The vitamins were pre-mixed using high-viscosity CMC (carboxymethyl cellulose) as the carrier. This mixture was extremely hygroscopic and also highly alkaline. This combination may have produced such conditions in the meal mixture that when it was fed with a meat supplement the vitamins were unavailable to the fish or were destroyed. Other possibilities are that the vitamin supplement was not adequate, or that a significant portion of the supplement was lost through leaching into the water.

Effect of protein levels

The original experiment was designed to test three protein levels, 20 percent, 25 percent, and 30 percent. However, from the start, the

Table 2:--Summary of 1962 feeding trials with chinook salmon, 24-week period

Initial number per tank: 406 fish
Initial weight per tank: 500 fish

Initial average weight per fish: 1.23 grams
Initial number per pound: 369 fish

Period: 2/7/62 - 8/21/62

Average water temperature: 53° F.

		Diet composition	Percentage composition	Calories per kg. of diet	Caloric ratio protein to energy	Average weight per fish (grams) 12 wks.	24 wks.	Percent mortality 12 wks.	24 wks.	Food conversion 12 wks.	24 wks.	Protein deposition (grams) 12 wks.	24 wks.	Protein utilization 12 wks.	24 wks.	Fat deposition (grams) 12 wks.	24 wks.	Performance Index 24 wks.
No vitamin supplement	20% protein	1. Leavenworth production	100.00	1400	1:0.68	7.10	18.45	1.72	10.8	2.82	3.08	376 .8	1148.8	3.73	3.60	175.4	378.1	32.5
No vitamin supplement	20% protein	2. Meat-meal ratio 50:50 Meat mix. A-1 meal mix. Water	 25.68 29.68 40.64	1308	1:0.63	7.80	21.58	1.60	47.0	2.59	4.62	438.7	1158.2	3.10	3.16	147.0	377.6	28.0
No vitamin supplement	20% protein	3. Meat-meal ratio 30:70 Meat mix. A-1 meal mix. Water Peanut oil	 15.23 35.53 49.13 .11	1300	1:0.62	7.30	21.58	0.37	60.1	2.68	6.47	413.2	988.0	3.28	3.46	133.4	288.6	28.5
No vitamin supplement	20% protein	4. Meat-meal ratio 20:80 Meat mix. A-1 meal mix. Water Peanut oil	 9.46 37.85 52.48 .21	1300	1:0.62	7.05	19.91	2.09	53.9	2.78	5.50	379.4	1017.2	3.38	3.40	125.2	273.6	23.3
No vitamin supplement	20% protein	5. Meat-meal ratio 10:90 Meat mix. A-1 meal mix. Water Peanut oil	 4.43 39.89 55.40 .28	1300	1:0.62	6.84	22.14	2.96	53.1	2.89	4.96	367.8	1063.6	3.45	3.17	125.3	316.0	33.0
Vitamin supplement	20% protein	6. Meat-meal ratio 10:90 Meat mix. A-1 meal mix. Water Peanut oil	 4.43 39.89 55.40 .28	1300	1:0.62	6.74	21.22	0.49	61.3	2.87	6.40	371.2	1038.2	3.44	3.22	120.0	282.8	27.0
Vitamin supplement	20% protein	7. Meat-meal ratio 10:90 Meat mix. A-1 meal mix. Water Peanut oil	 4.43 39.89 51.51 4.17	1650	1:1.06	8.09	21.08	0.86	13.8	2.43	2.94	446.9	1257.4	3.00	3.18	247.2	409.6	43.5
Vitamin supplement	20% protein	8. Meat-meal ratio 10:90 Meat mix. A-1 meal mix. Water Peanut oil	 4.43 39.89 47.62 8.06	2000	1:1.50	8.24	22.56	0.49	10.6	2.40	2.67	436.2	1307.6	3.12	3.15	314.6	750.2	43.0
No vitamin supplement	25% protein	9. Meat-meal ratio 50:50 Meat mix. A-1 meal mix. Water Peanut oil	 37.10 37.10 25.63 .17	1650	1:0.65	9.30	25.55	0.37	38.6	2.16	3.58	539.0	1438.3	3.27	3.56	224.6	484.4	27.5
No vitamin supplement	25% protein	10. Meat-meal ratio 30:70 Meat mix. A-1 meal mix. Water Peanut oil	 19.03 44.42 36.13 .42	1650	1:0.65	8.76	25.34	0.86	36.3	2.25	3.44	509.6	1450.6	3.34	3.48	191.1	434.7	28.2
Vit. supp.	25% protein	11. Meat-meal ratio 30:70 Meat mix. A-1 meal mix. Water Peanut oil	 19.03 44.42 32.24 4.31	2000	1:1.00	9.84	27.73	0.37	15.9	2.08	2.58	572.6	1671.2	3.16	3.36	320.0	699.5	34.5

Table 2 (continued):—Summary of 1962 feeding trials with chinook salmon, 24-week period

Initial number per tank: 406 fish
Initial weight per tank: 500 grams

Initial average weight per fish: 1.23 grams
Initial number per pound: 369 fish

Period: 2/17/62 - 8/21/62

Average water temperature: 53° F.

			Diet composition	Percentage composition	Calories per kg. of diet	Caloric ratio protein to energy	Average weight per fish (grams)		Perceot mortality		Food conversion		Protein deposition (grams)		Protein utilization		Fat deposition (grams)		Performance index 24 wks.
							12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.	
Vit. supp.	25% protein	12.	Meat-meal ratio 30:70 Meal mix. A-1 meal mix. Water Peanut oil	 19.03 44.42 28.35 8.20	2350	1:1.35	9.64	30.28	0.86	4.8	2.09	2.11	558.2	1855.2	3.16	3.14	356.4	1061.2	43.8
No vitamin supplement	25% protein	13.	Meat-meal ratio 20:80 Meat mix. A-1 meal mix. Water Peanut oil	 11.83 47.31 40.33 .53	1650	1:0.65	9.02	27.23	0.37	30.5	2.17	3.00	533.4	1543.3	3.20	3.32	215.0	453.4	28.0
No vitamin supplement	25% protein	14.	Meat-meal ratio 10:90 Meat mix. A-1 meal mix. Water Peanut oil	 5.54 49.86 43.97 .63	1650	1:0.65	8.14	25.74	0.62	43.2	2.36	3.68	466.8	1401.6	3.52	3.40	181.4	494.2	38.8
Vitamin supplement	25% protein	15.	Meat-meal ratio 10:90 Meat mix. A-1 meal mix. Water Peanut oil	 5.54 49.86 43.97 .63	1650	1:0.65	8.33	24.98	0.37	30.9	2.31	3.15	484.0	1453.6	3.42	3.43	200.2	471.8	37.0
Vitamin supplement	25% protein	16.	Meat-meal ratio 10:90 Meat mix. A-1 meal mix. Water Peanut oil	 5.54 49.86 40.08 4.52	2000	1:1.00	8.76	25.45	0.37	19.5	2.25	2.72	504.0	1489.8	3.39	3.51	288.0	767.2	37.5
Vitamin supplement	25% protein	17.	Meat-meal ratio 10:90 Meat mix. A-1 meal mix. Water Peanut oil	 5.54 49.86 36.19 8.41	2350	1:1.35	9.42	28.51	0.49	7.6	2.15	2.28	536.8	1685.4	3.30	3.38	363.4	949.6	42.0
Vitamin supplement	25% protein	18.	Meat-meal ratio 0:100 A-1 meal mix. Water Peanut oil	 52.09 43.31 4.60	2000	1:1.00	8.96	25.28	0.25	4.8	2.18	2.39	529.5	1538.0	3.22	3.55	310.1	692.0	43.2
Vitamin supplement	27.5% protein	19.	Meat-meal ratio 30:70 Meat mix. A-1 meal mix. Water Peanut oil	 20.94 48.86 27.68 2.52	2000	1:0.82	9.72	29.16	0.37	14.8	2.12	2.43	579.0	1794.6	3.46	3.49	306.5	634.6	41.8
Vitamin supplement	27.5% protein	20.	Meat-meal ratio 10:90 Meat mix. A-1 meal mix. Water Peanut oil	 6.09 54.85 36.31 2.75	2000	1:0.82	9.59	28.33	0.37	12.2	2.16	2.41	568.4	1720.2	3.48	3.64	283.7	712.2	37.0
Vitamin supplement	27.5% protein	21.	Meat-meal ratio 10:90 Meat mix. A-1 meal mix. Water Peanut oil	 6.09 54.85 32.42 6.64	2350	1:1.14	9.86	31.32	0.62	4.3	2.12	2.09	583.3	1940.1	3.48	3.39	364.4	1014.6	41.5
Least difference at the 5% confidence level:							0.56	5.01	0.42	29.2			46.4	97.6	0.30	0.25	36.6	226.5	12.52

Table 3.--Effect of meat on protein deposition and utilization and on mortalities

Diet number	Percent protein	Calories per kg. diet	Meat-meal ratio	Protein deposited (grams) 24 wks.	Protein utilization 24 wks.	Percent mortality 24 wks.
11	25.0	2,000	30:70	1,671	3.36	15.9
16	25.0	2,000	10:90	1,489	3.51	19.5
12	25.0	2,350	30:70	1,855	3.14	4.8
17	25.0	2,350	10:90	1,685	3.38	7.6
19	27.5	2,000	30:70	1,795	3.49	14.8
20	27.5	2,000	10:90	1,720	3.64	12.2

Table 4.--Effect of vitamin supplementation on protein deposition and utilization and on mortalities

Diet number	Percent protein	Calories per kg. diet	Meat-meal ratio	Vitamin supplementation	Protein deposited (grams) 24 wks.	Protein utilization 24 wks.	Percent mortality 24 wks.
5	20	1,300	10:90	No vitamin supplement	1,064	3.17	53.1
6	20	1,300	10:90	Vitamin supplement	1,038	3.22	61.3
14	25	1,650	10:90	No vitamin supplement	1,402	3.40	43.2
15	25	1,650	10:90	Vitamin supplement	1,454	3.43	30.9

fish receiving diets with 30 percent protein would consume only a part of the food present at each feeding. In order to prevent this loss, the protein level of these diets was lowered to 27.5 percent. No further trouble was encountered with the fish refusing food.

12 weeks.--The 25-percent protein diets produced significantly more weight and greater protein deposition than comparable diets fed at the 20-percent level of protein intake. There was no difference in protein utilization. The 27.5-percent protein diets, when compared with diets of 25 percent protein with comparable caloric intake and meat supplementation, produced fish which had significantly better protein deposition and equal protein utilization.

24 weeks.--Results at the end of the experiment were similar to those at 12 weeks. The 25-percent protein diets produced fish with significantly greater protein deposition than did comparable diets at the 20-percent protein level. Protein utilization factors did not differ significantly between comparable diets. In turn the 27.5-percent protein diets produced significantly greater protein deposition than did comparable diets at levels of 25 percent protein. There was no difference in protein utilization. Table 5 summarizes the results of comparable diets at the 25- and 27.5-percent protein levels at the end of 24 weeks.

These results would indicate that under these experimental conditions and at a constant water temperature, diets that provided a protein intake of 27.5 percent were superior to the comparable diets that supplied 20 and 25 percent protein. However, there is a trend, though not statistically significant, for the protein utilization factors at the 27.5-percent protein level

Table 5.--Effect of protein level on the protein deposition and utilization

Diet number	Percent protein	Calories per kg. diet	Meat-meal ratio	Protein deposited (grams) 24 wks.	Protein utilization 24 wks.
11	25.0	2,000	30:70	1,671	3.36
19	27.5	2,000	30:70	1795	3.49
16	25.0	2,000	10:90	1490	3.51
20	27.5	2,000	10:90	1720	3.64
17	25.0	2,350	10:90	1685	3.38
21	27.5	2,350	10:90	1940	3.39

to be higher than for comparable diets at the 25-percent level, indicating the possibility that the optimum efficiency level has been reached or slightly exceeded.

Effect of caloric levels

Peanut oil was added to seven diets in order to determine the effect of increased caloric intake on protein deposition, protein utilization, and fat deposition. Fish in diets 6, 7, and 8 were fed at a meat-meal ratio of 10:90 with caloric levels of 1,300, 1,650, and 2,000 calories per kilogram, respectively, and the protein intake was stabilized at 20 percent. At the 25-percent protein level, fish in diets 10, 11, and 12 were fed at a meat-meal ratio of 30:70, and their respective caloric intakes were 1,650, 2,000, and 2,350. The 10:90 meat-meal ratio in the 25-percent protein diets was also tested with fish in diets 15, 16, and 17 having caloric intakes of 1,650, 2,000, and 2,350. At the 27.5-percent protein level, fish in diet 20 were fed at a caloric intake of 2,000 calories per kilogram of food, while the caloric intake of fish in diet 21 was increased to 2,350 calories per kilogram. The results of this section are presented in table 6.

Protein deposition and utilization, 12 weeks. --At the 20-percent protein level, fish fed diet 7, at a caloric intake of 1,650 calories per kilogram of diet, had significantly greater protein deposition and utilization than did fish fed diet 6, at a caloric intake of 1,300 calories per kilogram of food. An additional increase to 2,000 calories, in diet 8, produced results no better than those of the 1,650-calorie diet. These results confirm those of Combs et al. (1962). Essentially the same results were found at the 25-percent protein level in the 30:70 meat-meal diets with fish from the 2,000-calorie ration, diet 11, and the 2,350-calorie ration, diet 12, having significantly greater protein deposition than fish from the 1,650-calorie ration, diet 10, but with no difference in protein utilization. The increase to 2,350 calories in diet 12 produced no significant increase in protein deposition when compared with diet 11, with 2,000 calories per kilogram. The 2,350-calorie ration, diet 17, in the 10:90 meat-meal ratio was the only diet in which the fish had a significant increase in protein deposition due to caloric increase. An increase in the caloric intake of fish in the 27.5-percent protein diets did not alter protein deposition or utilization.

24 weeks.--The analysis of the 20-percent protein diets at 12 weeks revealed that a significant increase in protein deposition and utilization occurred when the caloric intake of the fish was increased from 1,300 to 1,650 calories per kilogram, but increasing the caloric intake to 2,000 did not increase protein deposition or utilization above that of the 1,650-calorie diet. Results were the same at 24 weeks, with fish from diet 8 at 2,000 calories and diet 7 at 1,650 calories having significantly higher protein deposition than fish from the comparable diet 6 at 1,300 calories. There was no difference between fish in diets 8 and 7. At the 25-percent protein

Table 6.--Effect of caloric levels on protein deposition and utilization, fat deposition, and mortality

Diet no.	Percent protein	Calories per kg. diet	meat-meal ratio	Protein deposited (grams)		Protein utilization		Fat deposited (grams)		Percent mortality	Caloric protein to energy
				12 wks.	24 wks.	12 wks.	24 wks.	12 wks.	24 wks.	24 weeks	
6	20.0	1,300	10:90	371	1,038	3.44	3.22	120	283	61.3	1:0.62
7	20.0	1,650	10:90	447	1,257	3.00	3.18	247	410	13.8	1:1.06
8	20.0	2,000	10:90	436	1,308	3.12	3.15	315	750	10.6	1:1.50
10	25.0	1,650	30:70	510	1,451	3.34	3.48	191	435	36.3	1:0.65
11	25.0	2,000	30:70	573	1,671	3.16	3.36	320	700	15.9	1:1.00
12	25.0	2,350	30:70	558	1,855	3.16	3.14	356	1,061	4.8	1:1.35
15	25.0	1,650	10:90	484	1,454	3.42	3.43	200	472	30.9	1:0.65
16	25.0	2,000	10:90	504	1,490	3.39	3.51	288	767	19.5	1:1.00
17	25.0	2,350	10.90	537	1,685	3.30	3.38	363	950	7.6	1:1.35
20	27.5	2,000	10:90	568	1,720	3.48	3.64	284	712	12.2	1:0.82
21	27.5	2,350	10:90	583	1,940	3.48	3.39	364	1,015	4.3	1:1.14

level, protein deposition was significantly higher in fish from the 2,350-caloric diets than in fish from the 2,000-calorie diets at the end of 24 weeks. There was no difference in protein utilization. At 12 weeks, the 2,350-calorie diets could not be shown to be superior to the 2,000-calorie diets. While there was no difference at 12 weeks between fish from the 2,000-and 2,350-calorie diets at the 27.5-percent protein level, a significant increase in protein deposition and utilization was evident in fish from the 2,350-calorie ration, diet 21, as compared with fish from the 2,000-calorie ration, diet 20, at the end of 24 weeks. A sparing action on the protein requirements at higher caloric levels is indicated.

The increased caloric intake also had an influence upon mortalities. Mortality rates were significantly lower in the high-calorie rations, diets 7 and 8, than in the comparable low-calorie ration, diet 6, at the 20-percent protein level. Fish in diet 12 had a significantly lower mortality rate than did fish in diet 10 at the 25-percent protein level. Mortalities were attributed to a hypovitaminosis which became evident in the low-calorie, low-protein diets at 16 weeks and progressively spread into the high-protein, high-calorie diets until at the end of 24 weeks all of the diets, with the exception of the all-meat control diet, had some fish exhibiting the deficiency syndrome.

The degree of the hypovitaminosis was correlated with the levels of protein and calories fed, with the low-calorie, low-protein diets affected the most. A sparing action on the vitamin requirements at higher protein and caloric levels is indicated.

Fat deposition and protein calorie to energy calorie relation, 12 weeks.--An increase in caloric intake from 1,650 calories in diet 7 to 2,000 calories in diet 8 did not increase protein deposition or efficiency but did increase significantly the fat deposition of fish fed at the 20-percent protein intake level. A caloric intake level of 1,650 calories per kilogram with a protein calorie to energy calorie ratio of 1:1 appeared to be near optimum. These results were also similar to those of Combs et al. (1962). An increase in caloric level from 2,000 calories in diet 11 to 2,350 calories in diet 12 did not increase fat deposition, nor did it increase protein utilization of the fish fed at the 25-percent level of protein intake. Fish from both diets 11 and 12 had significantly higher fat and protein deposition than did fish from diet 10 at the 1,650-calorie level. An increase in calories from 2,000 in diet 16 to 2,350 in diet 17 did produce a significant increase in the fat deposition in the fish, but it did not increase the protein deposited. Here again a 1:1 ratio of protein calories to energy calories appeared to be optimum. In the 27.5-percent protein diets, an increase in the caloric intake of the fish from 2,000 in diet 20 to 2,350 in diet 21 significantly increased fat deposition but did not increase protein utilization or deposition. The protein calorie to energy calorie ratio was lower than the ratios in the 20- and 25-percent protein diets as would be expected since the protein intake was increased. The optimum level at this time appeared to be 1:0.8.

24 weeks.--Results at the 20-percent level of protein intake showed that diet 8 with 2,000 calories produced fish with significantly greater fat deposition than did diet 7 with 1,650 calories and diet 6 with 1,300 calories. There was no difference between fish from diets 7 and 6 in fat deposition. Raising the caloric level from 1,300 in diet 6 to 1,650 calories in diet 7 resulted in a significant increase in the amount of protein deposited in the fish. The additional increase to 2,000 calories in diet 8 did not increase protein deposited over that of the 1,650-calorie diet. A caloric intake of 1,650 calories per kilogram with a protein calorie to energy calorie ratio of 1:1 appears to be optimum as it did at 12 weeks. Increasing the caloric level from 1,650 calories in diets 10 and 15 to 2,350 calories in diets 12 and 17 increased fat and protein deposition with the protein utilization factor remaining the same. The increase to 2,350 calories also produced significantly greater protein deposition than did comparable diets at caloric intakes of 2,000. A protein calorie to energy calorie ratio of 1:1.35, with a caloric intake level of 2,350, appears to be superior to the 1:1 ratio favored at 12 weeks. Results at the 27.5-percent protein level were similar to those of the 25-percent level. The caloric increase to 2,350 in diet 21 produced significant increases in fat and protein deposition as compared with diet 20 at 2,000 calories. Whereas the 1:0.8 protein calorie to energy calorie ratio and a caloric intake at 2,000 appeared to be optimum at 12 weeks, a 1:1 ratio with a caloric intake level of 2,350 calories appears to be more favorable at 24 weeks, when feeding at a level of 27.5 percent protein.

Proximate analysis of samples of fish from the diets showed a reduction in the percentage of body fat in the fish at the end of 24 weeks as compared with analysis at the end of 12 weeks, while the percent protein in the body of the fish remained approximately the same. These changes in body composition indicate an increased energy requirement in the larger fish.

By increasing the caloric level of a diet, a sparing action was produced on the protein and vitamin requirements of the fish at all three levels of protein intake. Phillips et al. (1963), in diet trials with brook trout, also reported a sparing action on the protein utilization of brook trout, when corn oil was added to the diet. This sparing action is also in agreement with the results of Combs et al. (1962). At the 20-percent protein intake levels, a point appears to have been reached where further additions of calories as an energy source becomes inpractical. A protein calorie to energy calorie ratio of 1:1 was optimum at both 12 and 24 weeks. However, there was a trend towards greater protein deposition, as the ratio was increased to 1:1.5 in diet 8. The presence and extent of the hypovitaminosis may have been a factor which limited protein deposition at this protein level. At both the 25- and 27.5-percent protein levels, the protein calorie to energy calorie relation changed as the fish became older and larger, indicating an increased energy requirement. The increased protein deposition and utilization in the fish receiving these caloric increases supports this conclusion.

Stamina tests

The summarized results in table 2 show that the performance indices of the diet fish varied considerably. Normally, a difference in performance of three is considered to be significant. This wide spread can be explained by the extreme differences found within the diets themselves. Not all of the fish within a diet suffered the nutritional deficiency to the same degree; consequently, performance within the same diet was somewhat variable. Even though differences were not significant the fish in diets in which the vitamin deficiences were marginal had higher stamina indices than the fish in diets where the deficiency was extensive.

Vitamin injection studies

At the end of 16 weeks the fish in the low-calorie, low-protein diets began showing nutritional deficiencies. These deficiencies were characterized by frayed fin membranes, in which the upper extremities of the fin rays became bare and frayed; extreme emaciation due to a loss of appetite; partial retraction of the head; high mortalities; and a very noticeable loss of equilibrium, in which the fish swam at an angle from the horizontal with the head elevated. As the deficiency increased this head-up position became progressively worse, until the fish were nearly vertical and helplessly drifting with the current.

Fish in all of the diets, with the exception of the all-meat control, eventually showed this deficiency syndrome, despite high levels of meat and/or crystalline vitamin supplementation in some rations. The deficiency symptoms were believed to be a hypovitaminosis of one or more of the B-complex vitamins. In an attempt to determine which of the vitamins was lacking, an experiment was designed in which affected fish were injected with various B-complex vitamins, singly and in groups, in amounts equalling a 10-day requirement. Twenty days and two injections later, no noticeable alleviation of the deficiencies could be noted in any of the groups with the exception of the lot receiving the entire group of vitamins. In this group, the majority of the fish returned to a normal swimming position and began to feed readily. A chi-square test on mortalities showed only the lot receiving the complete vitamin complement to have a significantly lower mortality than the control.

The injection studies clearly indicated that the nutritional deficiency evident in the fish at the end of 24 weeks was due to an inadequate supply of the B-complex vitamins available to the fish. Prior calculation of the meal and meat combinations within the diets, showed that these ingredients were under the minimal requirements for chinook salmon as defined by Halver (1957). It was hoped that by supplementing the diets with a crystalline vitamin package containing the full amount of the B-complex group required for maximum storage that the requirements would be met. The appearance of the nutritional deficiency in all diets suggests that either the vitamin supplement was not adequate, or that a significant portion of the crystalline vitamins was unavailable to the fish either through loss by leaching into the water or an antagonistic action within the diets containing both meats and meals, or possibly a combination of all three factors.

An all-meal diet

Probably the most significant result of this experiment was the capability of an all-meal diet, devoid of raw meat supplementation and fed at 2,000 calories per kilogram, to maintain fish for a 24-week period. Gains, protein deposition and utilization, performance, and general condition were equal to if not better than comparable meat-supplemented diets. A cursory gill check was run on all diets every 2 weeks, and it was not until the end of 20 weeks that any fish from this diet were found to have anemic tendencies using pale gill color as the criterion. Mortalities were below 5 percent for the entire 24-week period and the nutritional deficiency so apparent in other diets was nearly lacking in this diet. Additional testing with this diet is indeed warranted.

SUMMARY

The results of the 1962 feeding trials may be summarized as follows:

1. At the end of the feeding trials, all of the experimental diets had some fish showing symptoms of a hypovitaminosis. The severity of these symptoms was most pronounced in the low-protein, low-calorie diets.

2. All experimental diets, with the exception of the 20-percent protein low-calorie rations, produced gains comparable to or exceeding that produced by the control diet.

3. Fish fed meat supplements ranging from 10 to 50 percent of the meat-meal combination were not measurably different in either mortality, average weight, protein deposition, or protein utilization.

4. A crystalline vitamin supplement had no measurable effect on either growth or survival in the meat-supplemented diets.

5. Diets fed at the 27.5-percent level of protein intake produced fish with higher levels of protein deposition than did diets fed at the 25- and 20-percent protein levels. Protein utilization factors did not differ significantly between comparable 27.5-percent protein diets and 25-percent protein diets, but the 27.5-percent protein diets tended to be less efficient.

6. Increasing the energy calorie intake of the fish at all three protein levels significantly increased the protein deposition without decreasing the protein utilization, and reduced both mortality and the symptoms of hypovitaminosis. A sparing action, at higher caloric intakes, on both the protein and vitamin requirements is indicated.

7. A protein calorie to energy calorie ratio of 1:1 appears to be optimum at the 20-percent protein level. The optimum protein calorie to energy calorie ratio increased at both 25-percent and 27.5-percent protein levels as the fish increased in age and size. A ratio of 1:1 appeared optimum in the 25-percent protein diets at 12 weeks, but at the conclusion of the experiment, a ratio of 1:1.35 appeared to be superior. Similarly at the 27.5-percent level of protein intake, a ratio of 1:0.8 was optimum at 12 weeks, but a ratio of 1:1 was superior at 24 weeks. Proximate analysis of the fish showed that the percentage of body fat decreased during the last 12 weeks of the experiment, while the percentage of body protein remained essentially the same. A higher energy requirement in the larger fish is indicated.

8. Stamina tests of the diet fish at the end of the feeding trials were not conclusive owing to the hypovitaminosis which caused a variable performance within the diets themselves.

Nutritional deficiency determination by means of vitamin injection indicated either that the fish were receiving an insufficient supply of vitamins or that the vitamins supplied were unavailable because of loss in the water or an antagonistic effect within the diet.

10. Gains, protein deposition, protein utilization, performance, and general condition of fish fed an all-meal diet were as good as or better than comparable diets fed with meat supplements.

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