SUPPLEMENTATION OF ALL-VEGETABLE PROTEIN CHICK RATIONS

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By

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SUPPLEMENTATION OF ALL-VEGETABLE PROTEIN CHICK RATIONS

INTRODUCTION

The principle ingredients of poultry rations are the common cereal grains. These cereal grains are deficient in one or more of the essential amino acids as well as certain mineral and vitamin components. Therefore, it has been shown necessary to supplement all cereal rations if adequate growth and development is to be insured.

Various protein concentrates are used to supplement the cereal grains. The animal protein supplements commonly used are by-products of the fishing industry, of the packing house, and of the dairy industry. Plant proteins used include soybean meal, peanut meal, and cottonseed meal.

Since the basic protein requirement is an adequate intake of the various essential amino acids plus an amount of nitrogen to maintain nitrogen equilibrium, a combination of plant and animal protein has long been considered an acceptable source of protein. The amount and kind of plant and animal protein supplements used are dependent upon the principle ingredients of the ration and the age of the chicks as well as such economic factors as cost and availability.

A good poultry ration is considered one which of its total protein, about one fourth is from animal sources. Early in 1942, wartime shortages of animal protein became critical. Since there was an abundance of soybeans, soybean oil meal came into widespread use as a high protein supplement for chick starting and growing rations. These inadequate supplies of animal protein for use as feedstuffs have only been partially responsible for the trend toward using larger quantities of vegetable protein in poultry rations. The availability of such materials as synthetic riboflavin and fermentation by-products has made the need for milk by-products and other animal proteins, as carriers of the known vitamins, less demanding. It is advocated by many workers that it is now possible to prepare adequate poultry rations using only vegetable protein supplements and mineral and vitamin supplements in place of animal protein supplements. Because vegetable protein supplements are generally cheaper than animal proteins, use of all vegetable rations has an effect on economical production.

However, many investigators have shown that rations for chickens containing only materials of vegetable origin are inferior for growth, egg laying, and hatchability of the eggs produced than those containing supplements of animal proteins. It has not been possible to fully explain these inadequacies on the basis of the amino acid composition. The protein of soybean oil meal, for example, is of high biologic value. Mixed poultry rations containing only materials of vegetable origin can be made which closely approximate the essential amino acid requirement of the chick. Supplementation of such rations with concentrates of various natural materials at levels providing only a very small amount of additional protein has been shown to result in greatly enhanced rates of growth in young chicks and in improved hatchability of eggs produced by mature fowls.

REVIEW OF LITERATURE

Hammond and Titus[1] found that 35% soybean oil meal was required in a diet adequately supplemented with minerals and vitamins to support rapid growth in chicks. Sardine meal at a level of 2% improved this soybean oil meal diet to the extent that it was superior to a high quality positive control diet containing meat scrap and dried skim milk. Berry et al.[2] found that rations containing 32% soybean oil meal resulted in a decreased rate of growth when compared with rations containing 20% soybean oil meal, 12% corn gluten meal, and 3% meat and bone scrap. Brant and Carver[3] found that when soybean oil meal was included in the ration as the sole source of protein, the growth of the chicks was slower, and the efficiency of ration utilization less than when fish meal or meat scrap was added to the ration.

It was also reported by Wilgus and Zander[4] that soybean oil meal used as sole source of protein supplement did not produce satisfactory growth. With as little as 1.25% of meat and bone scrap added to the ration, however, growth was improved. Addition of 2.5% of meat and bone scrap produced optimum rates of gain.

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[1] Hammond, John C., and Harry W. Titus, "The Use of Soybean Oil Meal in the Diet of Growing Chicks." Poultry Science, 23 (January, 1944), 49-57.

[2] Berry, E. P., C. W. Carrick, Roy E. Roberts and S. M. Hauge, "Supplementary Effect of Corn Gluten Meal with Soybean Oil Meal". Poultry Science, 25 (September, 1946), 498-500.

[3] Brant, A. W., and J. S. Carver, "From One Day of Age Through the First Laying Year on Rations Containing Soybean Oil Meal as a Protein Supplement". Poultry Science, 26 (November, 1947), 598-603.

[4] Wilgus, H. S. Jr., and D. V. Zander, "The Quantity of Animal Protein required to supplement Soybean Oil Meal for Growth." Poultry Science, 24 (January, 1945), 41-45.

Heuser *et al.*[5] found that soybean oil meal as the only source of supplementary protein produced heavier chicks at eight weeks than rations supplemented with peanut or wheat germ meal, which in turn was better than cottonseed meal or corn gluten meal. Inclusion of 3% fish meal in each ration resulted in improvement in every case. These workers concluded that the effect of the animal protein was additive rather than supplementary and that the growth response was not due chiefly to amino acids.

Many workers have found that supplementation of all vegetable rations with known factors of the B-complex will improve but not completely correct the deficiencies of the diet. In contrast to most workers, Mishler *et al.*[6] reported that a corn and soybean oil meal ration appeared to supply adequate biotin, inositol, para-amino benzoic acid, and vitamins E and K. Good growth of chicks was obtained when a 55% corn and 40% soybean oil meal ration was supplemented with riboflavin, pantothenic acid, nicotinic acid, pyridoxine, vitamins A and D, and minerals. From these studies, the workers concluded that animal protein supplements were not essential for good chick growth.

Patton *et al.*[7] found, however, that on a diet composed largely of ground corn, soybean oil meal, minerals, and adequately supplied with known vitamins, chicks did need a supplement for maximum growth.

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[5] Heuser, G. F., L. C. Norris, and J. McGinnis, "Vegetable Protein Concentrates Fed Alone and in Combination With Soybean Oil Meal and Fish Meal as the Chief Supplementary Protein in Chick Starting Rations." Poultry Science, 25 (March, 1946), 130-136.

[6] Mishler, D. H., C. W. Carrick, Roy E. Roberts, and S. M. Hauge, "Synthetic and Natural Vitamin Supplements for Corn and Soybean Oil Meal Chick Rations." Poultry Science, 25 (September, 1946) 479-485.

[7] Patton, A. R., J. P. Marvel, H. G. Petering, and J. Waddell, "The Nutritional Significance of Animal Protein Supplements in the Diet of the Chick." Jour. Nutrition, 31 (April, 1946) 485-495.

Clandinin et al.[8] reported that the addition of methionine to a soybean oil ration improved it, and in one instance the addition of methionine made an adequate ration. They further stated that methionine and choline cannot be considered interchangeable, since 3 out of 4 rations were not improved by choline addition.

Marvel et al.[9] however, found that choline and methionine seemed to exert an interchangeable action in a diet consisting of corn and soybean oil meal. This ration already contains considerable amounts of choline and methionine, but when either was added, there was a marked improvement in growth. These investigators also found that a ration of corn, soybean oil meal, distillers dried solubles, supplemented with minerals and vitamins produced growth equal to that obtained from the practical control ration containing meat and bone scrap. Marvel and co-worker[10] also reported that distillers dried solubles added to a vegetable protein ration supplemented with vitamins and minerals produced chick growth equal to a ration supplemented with both dried buttermilk and meat and bone scrap.

Bird and Mattingly[11] report that there was a significant improvement in growth of chicks when 0.2% dl-methionine was added to a ration consisting of yellow corn, oats, wheat by-products, soybean oil meal, alfalfa leaf meal,

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[8] Clandinin, D. R., W. W. Cravens, J. G. Halpin, and E.B. Hart, "Supplementary Value of Methionine, Cystine and Choline in a Practical Soybean Oil Meal Starter Ration." Poultry Science, 25 (September, 1946), 509-516.

[9] Marvel, James A., C. W. Carrick, Roy E. Roberts, and S. M. Hauge, "The Supplementary Value of Choline and Methionine in a Corn and Soybean Oil Meal Chick Ration." Poultry Science, 23 (July, 1945) 294-297.

[10] Marvel, James A., C. W. Carrick, Roy E. Roberts, and S. M. Hauge, "A Comparison of Soybean Oils and Soybean Oil Meals in Chick Rations Containing Distillers' Dried Solubles." Poultry Science, 24 (January, 1945), 46-52.

[11] Bird, H. R., and J. P. Mattingly, "Addition of Dl-Methionine to Starting and Growing Mashes." Poultry Science 24 (January, 1945), 29-33.

molasses butyl fermentation solubles, vitamins A and D, and mineral supplements. The growth stimulus of this ration slightly exceeded that obtained by a diet supplemented with 4% fish meal.

Novak, Hague, and Carrick[12] concluded from a series of feeding experiments that distillers dried solubles and condensed fish solubles contain an unidentified growth factor or factors which is necessary for chick growth.

Craven _et al._[13] report that a diet of yellow corn, wheat by-products, meat scrap, soybean oil meal, minerals, riboflavin, and fish oils was an unsatisfactory diet for growing chicks. Addition of 5% alfalfa leaf meal failed to significantly improve growth. There was only a slight improvement when 5% dried skim milk was added. Addition of condensed fish press water or ground fish viscera proved to be highly effective in supplementing the basal rations. A combination of dried skim milk and condensed fish press water resulted in the greatest growth stimulus.

Several workers have reported the effectiveness of cow manure added to an all vegetable ration for chicks. Hammond[14] in 1942 reported that cow manure had a marked beneficial effect on chick growth. Whitson et al.[15]

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[12] Novak, A. F., S. M. Hauge, and C. W. Carrick, "An Unidentified Growth Factor in Distillers' Dried Solubles Essential for the Chick." _Poultry Science_, 26 (November, 1947), 604-609.

[13] Cravens, W. W., W. H. McGibbon, and J. G. Halpin, "The Value of Certain Supplements in Practical Chick Rations Containing Adequate Riboflavin." _Poultry Science_, 24 (July, 1945), 305-309.

[14] Hammond, J. C., "Cow Manure as a Source of Certain Vitamins for Growing Chicks." _Poultry Science_, 21 (November, 1942), 554-559.

[15] Whitson, D., J. C. Hammond, H. W. Titus, and H. R. Bird, "The Use of Soybean Meal in the Diet of Growing Chicks." II "The Effect of Different Grains." _Poultry Science_, 24 (September, 1945), 408-416.

found that cow manure exerted a growth stimulus when added to a ration of corn and soybean oil meal with vitamin and mineral supplements. Feeding experiments conducted by these investigators indicate that the growth promoting factor is not one of the known vitamins. Rubin and Bird[16] have reported that the chick growth factor in cow manure is not identical with the previously described factors. These workers have prepared an ethanol soluble extract from cow manure that is effective in promoting chick growth.[17]

Rubin, Bird, and Rothchild[18] report that feces of hens added to a corn and soybean oil meal diet promoted growth in chicks. This may be due to intestinal synthesis of an unidentified factor.

Recently, feeding experiments have been carried on using fish solubles as a source of the growth promoting factor. Grau[19] found that 5% fish solubles added to a diet composed of soybean oil meal, alfalfa, mineral and vitamin supplements greatly stimulated the growth of chicks. The effect of the fish solubles did not appear to be due to the protein content. Lassen and Bacon[20] found that as high as 12 or 13% fish solubles could be added to the ration with good results.

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16 Rubin, Max, and H. R. Bird, "A Chick Growth Factor in Cow Manure. I. Its Non-identity With Chick Growth Factors Previously Described." Jour. Biological Chem., 163 (1946), 387-392.

17 Rubin, Max, and H. R. Bird, "Chick Growth Factor in Cow Manure. II. The Preparation of Concentrates and the Properties of the Factor." Jour. Biological Chem., 163 (1946), 393-400.

18 Rubin, Max, H. R. Bird and Irving Rothchild, "A Growth Promoting Factor for Chicks in the Feces of Hens." Poultry Science, 25 (September, 1946), 526-528.

19 Grau, C. R., "Fish Solubles for Feeding Poultry." Flour and Feed, 47 (November, 1946), 46.

20 Lassen, Sven, and E. Kyle Bacon, "The Use of Condensed Fish Solubles in Poultry Nutrition." Poultry Science, 25 (1946) 486-491.

Mishler, Carrick, and Hauge[21] also used fish solubles as a supplement for corn and soybean oil meal rations and found that a level of 36% soybean oil meal, 58% corn, 1.5% fish solubles with added minerals and vitamins showed best growth. When a basal ration was supplemented with fish solubles and riboflavin, and compared with one supplemented with 4 synthetic vitamins and methionine without fish solubles; the results gave a highly significant difference in favor of fish solubles and riboflavin.

Nichols, Robblee, Cravens, and Elvehjem[22] report that an ethanol insoluble fraction of fish solubles added to a basal ration at a level equivalent to 3% fish solubles resulted in a distinct growth response. These workers also found that the addition of .05 cc. of reticulogen, an anti-pernicious anemia factor, resulted in a growth response equal to that of a ration containing fish solubles.

These workers also report[23] that the unidentified factor exerts a growth stimulus not only with diets composed of plant protein but with purified diets as well. This growth response is used as a method of bioassay in the fractionation of the unidentified factor or factors in fish solubles. They further report that the active factor in fish solubles is insoluble in 95% ethanol, acetone, or ether; soluble in water and 70% methanol, the higher the concentration of alcohol, the less soluble the factor is.

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21 Mishler, D. H., C. W. Carrick, and S. M. Hauge, "Condensed Fish Solubles as a Supplement for Corn and Soybean Oil Meal Chick Rations." Poultry Science, 26 (September, 1947) 550-551 (abstract).

22 Nichol, C. A., A. E. Robblee, W. W. Cravens, C. A. Elvehjem, and "Activity of Anti-pernicious Anemia Preparations in Chicks. Jour. Biol. Chem., 170 (September, 1947) 419-420.

23 Robblee, A. R., C. A. Nichols, W. W. Cravens, C. A. Elvehjem, and J. G. Halpin, "Studies on an Unidentified Growth Factor in Fish Solubles." Poultry Science 26, (September, 1947), 553 (abstract).

McGinnis, Stevens, and Groves[24] state that the unidentified factor in liver is destroyed by oxidation, that it is soluble in water and insoluble in acetone, Darco failed to absorb it at pH of 5, 4, 3, 2, and the active fraction is dialyzable through a cellophane membrane. They found that methionine added to a chick basal ration didn't replace the factor, but gave additional growth response.

McGinnis and Carver[25] showed that the chicks requirement for the unidentified factor did not appear to be dependent upon the presence of corn or soybean oil meal in the chick diet. They[26] also state that the need for the factor is influenced markedly by the type of diet fed the breeders. When a ration containing a high level of the factor was fed to the hens, need for the factor could not be shown in the chicks.

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24 McGinnis, James, Joan M. Stevens, and Kermit Groves, "Studies on an Unidentified Factor Required for Chick Growth and Livability." Poultry Science, 26 (September, 1947), 550 (abstract).

25 McGinnis, James, and J. S. Carver, "Do Cereal Grains and Soybean Oil Meal Influence the Chick's Need for an Unidentified Factor or Factors Present in Fish Meal and a Liver Fraction. Poultry Science, 26 (September, 1947), 489-493.

26 McGinnis, James, and J. S. Carver, "The Storage of an Unidentified Growth Factor or Factors in the Egg and Its Relation to Chick Growth and Mortality. Poultry Science, 26 (September, 1947), 457-462.

EXPERIMENTAL

General

In all these studies, 12 or more day-old chicks were used per lot. They were wing-banded and distributed at random. Male chicks were used in all trials except trial 1, in which half were males and half were females. In trial 1, New Hampshire chicks were employed, in trials 2 and 5, Silver Oklabars, and in trials 3 and 4, Dominant Whites. Trials 1 and 2 were carried four weeks and trials 4 and 5 were carried six weeks each.

The chicks were housed in a thermostatically controlled electrically heated battery brooder, maintained at a temperature suitable for the age of the chicks. Food and water were provided ad lib. The chicks were weighed at weekly intervals. Instead of reporting the gain of each chick, the average gain in weight for the entire lot is recorded.

EXPERIMENT I: Vitamin and Amino Acid Supplementation of All-Vegetable Rations

Trial 1. It was felt desirable at the initiation of this study to attempt to duplicate under our conditions the observations of other workers. To this end a comparison of an all-vegetable protein ration, composed of grains from various sources and supplemented with minerals and riboflavin, was compared with a ration containing adequate amounts of the principle B-complex vitamins. This ration plus the B-complex vitamins was also supplemented with fish solubles.

In addition to the lots fed the mixed vegetable protein ration, groups supplemented in the same way were placed on a simple ration consisting of corn and soybean oil meal. All rations provided between 21.0% and 21.5% protein.

This experiment was also designed to study the effect of addition of various materials on the growth rates of chicks fed an all-vegetable protein ration and supplemented with minerals, riboflavin, and other principle members of the B-complex.

Folic acid, casein, alfalfa leaf meal, green feed, and fish solubles were tested for the effect each had on the growth rate of chicks. Composition of the rations is shown in Table I.

Lot 13 was fed a practical poultry ration containing animal protein as a supplement. Composition of this ration is shown in Table II. The average gains in weight of the chicks in trial 1 are shown in Table III.

The effect of the addition of dl-methionine and whey fermentation solubles on the growth of chicks was also tested. These materials were added to the rations used in trial 5. The composition of these rations is shown in Table IX. Since each gram of whey fermentation solubles contained 2500 micrograms of riboflavin, no additional riboflavin was used in this lot. The results of the addition of these materials are shown in Table IV.

Trial 2. The same rations and supplementations which were described in trial 1 were used in this trial. The results were essentially the same. Rates of gain were somewhat reduced but the relative position of the lots on the variously supplemented rations were the same. Average gains in weight of the chicks are shown in Table V.

Trial 3. Since the addition of the principle members of the B-complex gave enhanced rates of growth, it was thought desirable to attempt to find which of these vitamins were responsible for the stimulation. The basal

TABLE I.

COMPOSITION OF RATIONS USED IN TRIALS 1 AND 2.

Ingredient	Lot Numbers											
	1	2	3	4	5	6[1]	7	8	9[2]	10	11	12
	14	15	16	17	18	19	20	21	22	23	24	25
Ground yellow corn	28	28	28	32	36	36	43	35	36	57	57	57
Soybean oil meal	23	23	20	21	25	25	13	23	25	39	39	36
Ground wheat	15	15	15	15	15	15	15	15	15			
Corn gluten meal	10	10	10	10	10	10	10	10	10			
Pulverized barley	10	10	10	10	10	10	10	10	10			
Alfalfa leaf meal	10	10	10	5								
Steamed bonemeal	2	2	2	2	2	2	2	2	2	2	2	2
Calcium carbonate	1	1	1	1	1	1	1	1	1	1	1	1
Salt	1	1	1	1	1	1	1	1	1	1	1	1
Fish solubles			3					3				
Casein							5					
*Riboflavin Mix	3 gm.	3 gm.	3 gm.	3 gm.	3 gm.	3 gm.	3 gm.	3 gm.	3 gm.	3 gm.	3 gm.	3 gm.
**B-complex vitamins	−	+	+	+	+	+	+	+	+	−	+	+

Vitamin A and D feeding oil added to all lots at a level of 0.25%.

*Riboflavin mix: 1 gm. riboflavin per ounce of mix.
**Vitamins added per 100 gm. of ration: Thiamine 0.20 mg., pantothenic acid 1.10 mg., pyridoxine 0.35 mg., nicotinic acid 1.80 mg., choline 154.00 mg.

1 Folic acid added per 100 gm. of ration: 0.10 mg.

2 Oat sprouts and grass added ad. lib.

TABLE II.

COMPOSITION OF PRACTICAL POULTRY RATION

USED IN LOT 13

Ingredient	Percent
Ground yellow corn	61.00
Wheat shorts	5.00
Alfalfa Leaf meal	3.00
Soybean oil meal	5.00
Cottonseed oil meal	5.00
Dried buttermilk	15.00
Meat and bone scrap	5.00
Vitamin A and D feeding oil	0.25
Salt	0.75
TOTAL	100.00

TABLE III.

AVERAGE WEEKLY GAIN IN WEIGHT OF

CHICKS IN TRIAL 1

Lot Number	Av. Gain in Weight (gms.) per Week				
	1	2	3	4	Total
1	17	30	50	66	163
2	17	39	50	96	202
3	16	38	82	104	240
4	17	42	90	127	276
5	24	35	51	83	193
6	20	44	75	84	223
7	17	52	57	117	243
8	22	48	97	123	290
9	33	32	68	94	227
10	9	23	22	26	80
11	22	39	45	89	195
12	28	50	97	126	301
13	12	30	86	76	204

TABLE IV.

AVERAGE WEEKLY GAIN IN WEIGHT OF CHICKS ON VARIOUS SUPPLEMENTS

Lot	Rations and Supplementation	Av. Gain in Weight (gms.) Per Week						
		1	2	3	4	5	6	Total
43	Basal	17	29	47	57	90	56	296
44	Basal + Vitamins — Folic Acid	27	36	77	74	109	96	419
45	Basal + Vitamins	26	29	69	78	75	79	356
53	Basal	23	26	55	53	55	83	295
54	Basal + Vitamins	38	61	84	93	96	101	473
57	Basal + Vitamins + Methionine*	37	71	85	96	80	114	483
58	Basal + Vitamins + Whey Fermentation Solubles**	34	65	95	119	113	133	559
55	Basal + Vitamins + Fish Solubles***	39	75	127	118	152	158	659

* 1.54 gm. dl-methionine per 100 gm. feed.
** 3.0 gm. whey fermentation solubles per 100 gm. feed furnished 1% additional protein.
*** 3.0 gm. fish solubles per 100 gm. feed furnished 1% additional protein.

TABLE V.

AVERAGE WEEKLY GAIN IN WEIGHT OF

CHICKS IN TRIAL 2

Lot Number	Av. Gain in Weight (gms.) Per Week				
	1	2	3	4	Total
14	15	26	44	60	145
15	17	40	58	84	199
16	18	26	65	91	200
17	16	29	64	82	191
18	19	28	48	73	168
19	19	34	59	78	190
20	18	41	60	83	202
21	18	38	61	113	230
22	17	38	57	85	197
23	14	32	33	49	128
24	18	40	25	61	174
25	21	37	80	97	235
26	20	30	45	85	180

ration that was used is shown in Table VI. This ration was then supplemented with the following B-complex vitamins: (mg. per 100 gm. of feed) riboflavin 0.2, nicotinic acid 1.80, pantothenic acid 1.10, pyridoxine 0.35, folic acid 0.10, choline 154.00, thiamine 0.20, inositol 22.00, para-amino benzoic acid 1.10. Besides feeding one lot on the basal ration and one on the basal ration plus all the B-complex vitamins mentioned above, six other lots were tested. The diets for these lots consisted of the basal ration plus the B-complex vitamins minus (1) riboflavin (2) choline (3) folic acid (4) pantothenic acid (5) pyridoxine (6) nicotinic acid. The rates of growth of the chicks in each lot were determined and those data are presented in Figure 1. The average weight of the chicks is indicated by the slope of the line, each unit representing a gain of 10 gm. in weight. The average weekly weights of the chicks are shown in Table VII.

The growth rate of chicks on an all-vegetable ration supplemented with B-complex vitamins was compared with the growth rate of chicks on a normal starting ration containing animal protein as a supplement in trial 4. The composition of the normal starting mash is shown in Table VIII. Lot 43 was fed the basal ration shown in Table IX supplemented with minerals and riboflavin. Lot 45 was fed the basal ration supplemented with the B-complex vitamins. Lot 47 was fed the normal starting mash containing animal protein as a supplement. The results are shown in Table X.

A further study on the effect of folic acid on the growth rate of chicks was carried out in trial 4. Lot 44 was fed a basal ration (Table IX) supplemented with all the B-complex vitamins except folic acid. Lot 45 was fed the same basal ration supplemented with all the B-complex vitamins. Lot 43 was fed the basal ration supplemented only with riboflavin. Results of these studies are shown in Table IV.

TABLE VI.

COMPOSITION OF THE BASAL RATION

USED IN TRIAL 3

Ingredient	Percent
Ground yellow corn	57.0
Soybean oil meal	39.0
Calcium carbonate	1.0
Steamed bonemeal	2.0
Salt	1.0
TOTAL	100.0

Vitamin A & D feeding oil added at the level of 0.25%.

Figure 1. Growth rates of chicks on basal ration no. 2, minus various vitamins.

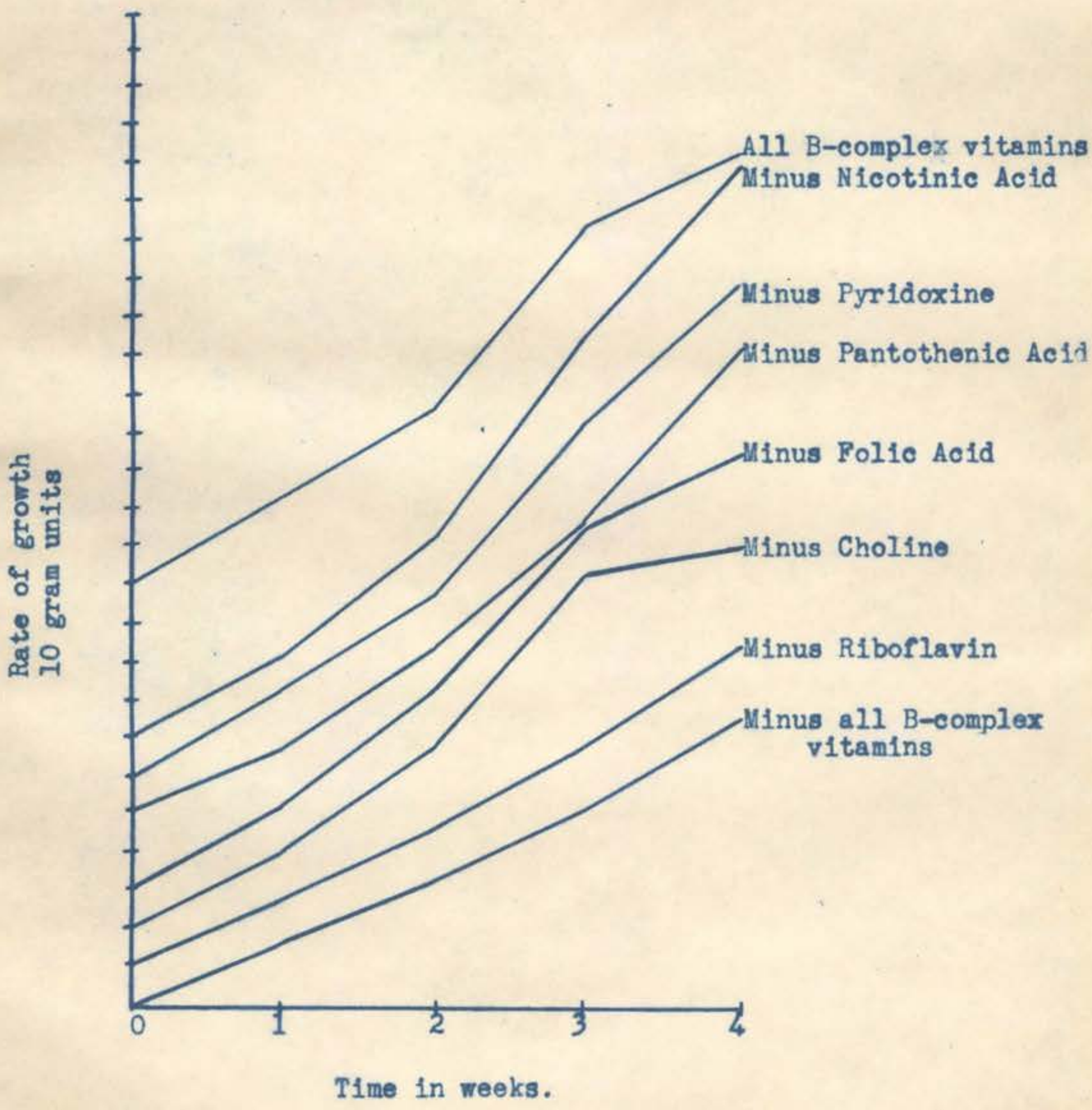


TABLE VII.

AVERAGE WEEKLY GAIN IN WEIGHT OF CHICKS IN TRIAL 3

Lot. No.	Supplementation	Av. Gain in Weight (gms.) Per Week				
		1	2	3	4	Total
27	Basal	35	29	39	46	149
28	B-complex vitamins	43	52	89	37	221
29	B-complex – Nicotinic acid	42	64	102	89	297
30	B-complex – Pyridoxine	43	54	90	70	257
31	B-complex – Pantothenic acid	37	47	70	87	241
32	B-complex – Folic acid	42	63	85	34	224
33	B-complex – Choline	41	55	92	11	199
34	B-complex – Riboflavin	36	36	45	51	168
35	B-complex + Fish solubles	46	69	115	100	330

TABLE VIII.

NORMAL STARTING MASH FED TO LOT 47

Ingredient	Percent
Ground yellow corn	24
Wheat shorts	20
Wheat bran	10
Pulverized barley	10
Alfalfa leaf meal	10
Dried buttermilk	5
Soybean meal	10
Meat and bone scrap	10
Salt	1
TOTAL	100

Vitamin A and D feeding oil added at the level of .2%.

EXPERIMENT II: Chemical Fractionation of Fish Solubles

The fish solubles used in these experiments is what is commonly known as fish press water, which is the body moisture, gastric juices, tissue particles etc. removed from the freshly pressed sardine fish oil. This is in turn reduced to a 50% solids consistency through a series of vacuum evaporations. The protein content of the fish solubles used is approximately 33%.

Chemical fractionation of the active material present in fish solubles was started, using various concentrations of alcohol. These alcohol fractions were added to a corn and soybean oil meal basal ration supplemented with minerals and B-complex vitamins. Composition of the basal ration and amounts of B-complex vitamins used in all of the testing of activity of fractions is shown in Table IX.

Trial 4. consisted of the separation of two different concentrations of alcohol and fish solubles. One mixture contained 50% alcohol and 50% fish solubles. The other contained 75% alcohol and 25% fish solubles.

1000 gms. of fish solubles and 1000 cc. of 95% alcohol were mixed together, making a 50% alcohol concentration. This was mixed with a stirring rod and centrifuged. The supernatant liquid, which was clear dark brown, was poured off and the residue extracted twice more with alcohol, 500 cc. being used each time. At the last extraction the supernatant liquid was light brown. The residue was discarded. The liquids were collected and reduced in volume under diminished pressure to approximately 500 cc. This substance was very dark brown and viscous. Absolute alcohol was poured into this, and as the mixture was stirred, a hard gummy mass

TABLE IX.

COMPOSITION OF THE BASAL RATION

USED IN TRIALS 4 AND 5.

Ingredient	Percent
Ground yellow corn	57.0
Soybean oil meal	39.0
Calcium carbonate	1.0
Steamed bonemeal	2.0
Salt	1.0
TOTAL	100.0

Vitamin A and D feeding oil added at the level of 0.25%.
Riboflavin mix added per 100 lbs. of feed: 3 gms.
Mg. of B-complex vitamins per 100 gms. of feed: thiamine 0.20, pantothenic acid 1.10, pyridoxine 0.35, nicotinic acid 1.80, choline 154.00, folic acid 0.10, inositol 22.00, para-amino benzoic acid 1.10.

was precipitated. The mass became harder, as it was extracted with absolute alcohol, and could be separated from the rest of the mixture. The solid portion was further extracted, using a mortar and pestle, until the precipitate was ground into a powder. This material was very hydroscopic, and was kept covered with absolute alcohol to keep it dry. This alcohol precipitate (Fraction I) was added to the basal ration in lot 36.

The portions of absolute alcohol that were used to extract the precipitate described above, were collected and 30 gm. of Norite A was added. This mixture was stirred, heated, and filtered on a suction pump. The filtrate was treated again with 20 gm. of Norite A. This norite filtrate (Fraction II) was added to the basal ration in lot 37.

To the norite residue left from the above process, alkaline alcohol (500 cc. alcohol and 50 cc. NH_4OH) was added. This mixture was stirred, heated, and filtered on a suction pump. The norite residue was treated again with alkaline alcohol. The liquids were collected, and this norite eluate (Fraction III) was added to the basal ration in lot 38.

The norite absorbate (Fraction IV) left from this process was added to the ration in lot 39. Figure 2 shows a summary of the fractionation.

1000 gms. of fish solubles and 3000 cc. of 95% alcohol were mixed together, making a 75% alcohol concentration. This mixture was stirred and centrifuged. The supernatant liquid was poured off, and the residue was extracted with 500 cc. of alcohol, and centrifuged twice more. The residue was spread in a pan and allowed to dry. It was broken into small pieces in a mortar and pestle, then ground to a fine powder in a mill. This alcohol insoluble residue (Fraction V) was added to the feed in lot 40.

1000 gms. Fish Solubles and 1000 cc. 95% alcohol

mixed and centrifuged.

Supernatant liquids reduced to 500 cc. and

extracted with 1000 cc. absolute alcohol.

Extracts combined and mixed with 50 gm. Norite A

and filtered

Alcohol Precipitate (fraction I)

Norite filtrate (fraction II)

Norite eluted with 500 cc. alkaline alcohol

Norite eluate (fraction III)

Norite absorbate (fraction IV)

Figure 2. A Summary of A Chemical Fractionation of Fish Solubles.

The supernatant liquids from the above process were collected, and reduced in volume under diminished pressure to approximately 500 cc. This viscous mixture was then treated with absolute alcohol as previously described. This alcohol precipitate (Fraction VI) was added to the basal ration in lot 46.

The portions of absolute alcohol used to precipitate fraction VI were collected and added (Fraction VII) to the basal ration in lot 42. Fraction VII contained the part of the fish solubles that was soluble in a concentration of 75% alcohol and also soluble in absolute alcohol.

One-half of each fraction was added per 20 pounds of feed. This was equivalent to approximately 5% fish solubles. The effect on the growth of chicks of adding these fractions is shown in Table X.

Trial 5. 2000 gms. of fish solubles and 4000 cc. of 95% alcohol, making approximately a 66% alcohol concentration, were mixed and stirred mechanically for an hour, then centrifuged. The supernatant liquid which was a clear, dark brown, was poured off and the residue again mixed with alcohol to a level of 66%, mechanically stirred and centrifuged again. This process was repeated two more times. The supernatant liquid at the last extraction was a clear, amber color. The residue which was a light brown color was spread in a pan and allowed to dry. It was ground in a mill, and added to the feed in lot 48. This fraction (Fraction VIII) was the portion of fish solubles that was insoluble in a 66% alcohol concentration.

The supernatant liquids were collected and reduced in volume under diminished pressure to approximately 500 cc. This dark, brown, viscous substance was poured into 3000 cc. of absolute alcohol and stirred.

TABLE X.

AVERAGE WEEKLY GAIN IN WEIGHT OF CHICKS IN TRIAL 4

Lot No.	Supplementation	Av. Gain in Weight (gms.) Per Week						
		1	2	3	4	5	6	Total
36	Alcohol precipitate fraction I*	34	42	83	110	138	163	570
37	Norite filtrate fraction II*	23	42	64	87	73	104	393
38	Norite eluate fraction III*	21	45	65	82	85	110	408
39	Norite absorbate fraction IV*	22	39	70	88	84	103	406
40	Alcohol insoluble fraction V**	29	37	64	105	96	97	428
41	Alcohol precipitate fraction VI**	24	42	75	82	130	154	507
42	Alcohol soluble fraction VII**	29	46	83	85	119	153	515
43	None	17	29	47	57	90	56	296
45	B-complex vitamins	26	29	69	78	75	79	356
46	B-complex vitamins, fish solubles	27	40	85	94	144	124	514
47	Normal starting mash	23	32	70	62	113	97	397

* Fractions made from mixture of 50% alcohol and 50% fish solubles.
** Fractions made from mixture of 75% alcohol and 25% fish solubles.

Lots 36 through 42 also supplemented with B-complex vitamins.

This was allowed to stand for a day, then the dark brown, clear liquid poured off, and the remaining thick, sticky residue was repeatedly extracted with small portions of absolute alcohol using a mortar and pestle until all the residue was ground to a light brown powder. This hydroscopic powder (Fraction IX) was added to the ration in lot 49.

The absolute alcohol used for extracting the alcohol precipitate was collected and reduced in volume under diminished pressure to about 400 cc. Crystals formed and the mixture was filtered. The filtrate was poured into 1500 cc. of absolute alcohol and stirred. The absolute alcohol precipitated only a very little material. This was allowed to stand overnight for the insoluble material to settle out. The mixture was decanted and the small amount of insoluble material clinging to the sides of the flask was discarded. One-half of this filtrate (Fraction X) was put aside to be used in the feed in lot 50. This filtrate contained the portion of fish solubles that was soluble both in 95% alcohol and absolute alcohol.

The other half of this filtrate was adjusted to the pH of 3 and treated with Norite A. 15 gms. of Norite A was added and the mixture was stirred, heated, and filtered. This process was repeated five times, using 15 gms. of Norite A each time. The filtrate was a clear, dark brown, but not quite as dark as the untreated filtrate. This norite filtrate (Fraction XI) was added to the feed in lot 51. The norite residue was eluted with alkaline alcohol three times. The norite eluate (Fraction XII) was light brown with a green tinge. This eluate was added to the basal ration in lot 52.

The norite residue (Fraction XIII) was added to the feed in lot 53. The results of adding the various alcohol fractions to the basal ration are shown in Table XI.

TABLE XI.

AVERAGE WEEKLY GAIN IN WEIGHT OF CHICKS IN TRIAL 5

Lot No.	Supplementation	Av. Gain in Weight (gms.) Per Week						
		1	2	3	4	5	6	Total
47	Alcohol insoluble fraction VIII*	39	73	113	121	119	161	626
48	Alcohol precipitate fraction IX*	38	69	96	114	121	147	585
49	Alcohol soluble fraction X*	32	58	75	78	71	130	444
50	Norite filtrate fraction XI*	28	60	93	90	106	124	501
51	Norite eluate fraction XII*	33	57	82	80	97	111	460
52	Norite absorbate fraction XIII*	36	55	76	59	49	42	317
53	None	23	26	55	53	55	83	295
54	B-complex vitamins	38	61	84	93	96	101	473
55	B-complex vitamins, fish solubles	39	75	127	118	152	158	659

*Fractions made from mixture of 66 2/3% alcohol and 33 1/3% fish solubles.
Lots 47 through 52 also supplemented with B-complex vitamins.

RESULTS AND DISCUSSION

One purpose of the experiment was to compare the rates of growth of chicks fed various mixed all-vegetable rations.

Lot 1 was fed a ration containing protein only from vegetable sources supplemented with minerals and riboflavin. Lot 2 was fed the same ration supplemented with the principle B-complex vitamins. In lot 3, the basal ration was supplemented with the B-complex vitamins, and in addition, 3% of the total ration was fish solubles, which replaced a like amount of soybean oil meal. The quantity of fish solubles represented 1% of the protein total.

At four weeks of age, lot 2 averaged approximately 25% more weight than lot 1, and lot 3, almost 50% more than lot 1. Figure 3 shows average gains in weight of chicks on these rations.

A second series of rations were used which contained only yellow corn and soybean meal, supplemented with minerals and riboflavin. Lot 10 was fed only this basal ration. Lot 11 was given the same ration with the addition of the principle B-complex vitamins. Lot 13 was supplemented with B-complex vitamins, and in addition, 3% of the total ration was fish solubles, which replaced a like quantity of soybean oil meal, thereby furnishing 1% of the protein total.

After the first four weeks of growth, lot 11 had an average weight approximately 2 1/2 times greater than lot 10. Lot 12 had an average weight approximately 3 2/3 times greater than lot 10. Figure 4 shows the average gains in weight of chicks on these rations.

Figure 3. Growth rates of chicks in trial 1 on mixed all-vegetable rations, supplemented with vitamins and fish solubles.

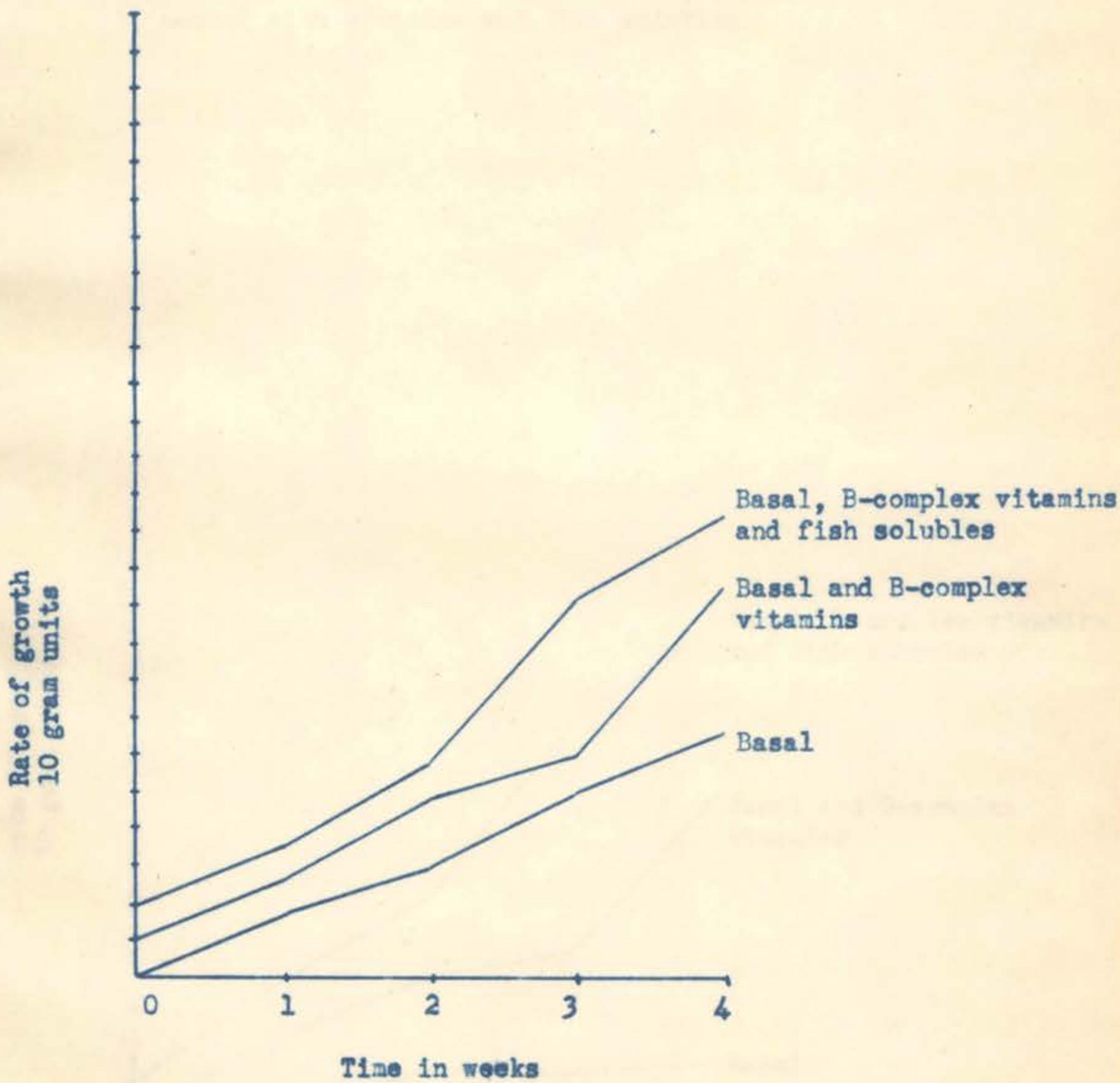
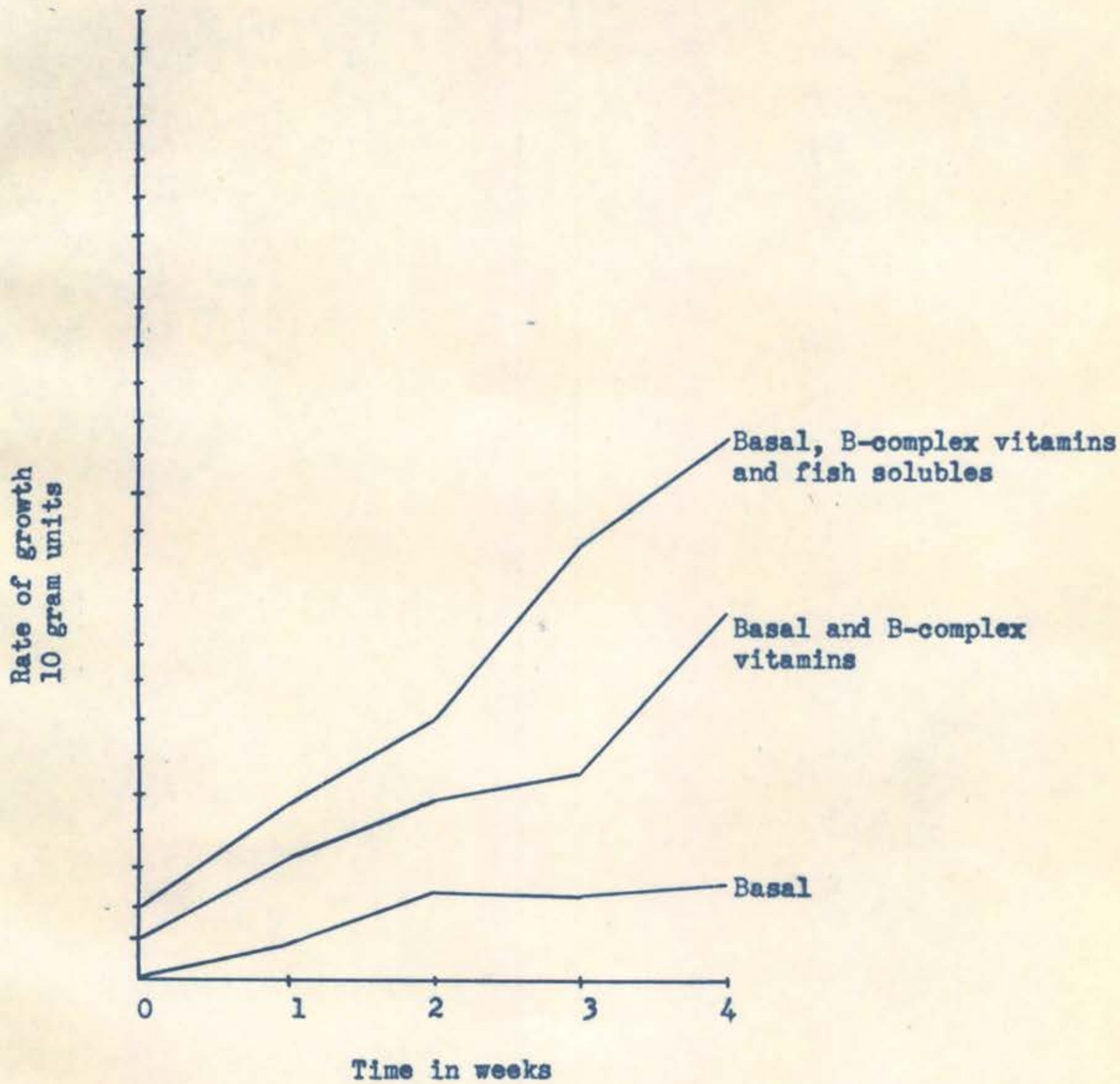


Figure 4. Growth rates of chicks in trial 1 on corn and soybean oil meal rations, supplemented with vitamins and fish solubles.



A comparison of lots 1, 2, and 3 with lots 10, 11, and 12 in trial 1, shows that the average weights of chicks on the mixed vegetable protein ration were greater than the average weights of those on the corn and soybean oil meal ration when supplements were not added. The provisions of an adequate level of the principle B-complex vitamins to both rations resulted in improved growth. Based on this experiment, the growth rates of the chicks on the mixed vegetable protein ration were only slightly superior to the growth rates of those on the corn and soybean oil meal ration. However, when supplemented with fish solubles, the soybean oil ration was distinctly superior to the mixed vegetable protein ration.

The differences between the growth rates of chicks on these two rations may be due to one or both of the following factors; (1) the presence in the mixed vegetable protein ration of greater amounts of the growth factor distributed in the various cereal grains would account for better growth rates of chicks on this ration in the absence of fish solubles; (2) the fact that soybean oil meal contains a high quality protein may account for the enhanced rate of growth when supplemented with B-complex vitamins and the growth factor present in fish solubles.

It would appear from these results that a corn and soybean oil meal ration, when properly supplemented with crystalline vitamins and an unknown factor present in fish solubles, is capable of supporting near maximum growth.

A further comparison of an all-vegetable ration with a ration containing animal protein was carried out in trial 4. The growth rate of the chicks in the lot fed a normal starting mash containing animal protein as a supplement was not significantly higher than the growth rate of chicks on an all-vegetable ration supplemented with B-complex vitamins. The gain in weight of the chicks in these two lots were practically the same. However, the growth rate of the lot fed an all-vegetable ration supplemented only with riboflavin was approximately 25% less than the growth rate of the chicks on the normal starting mash. Composition of the normal starting mash is shown in Table VIII.

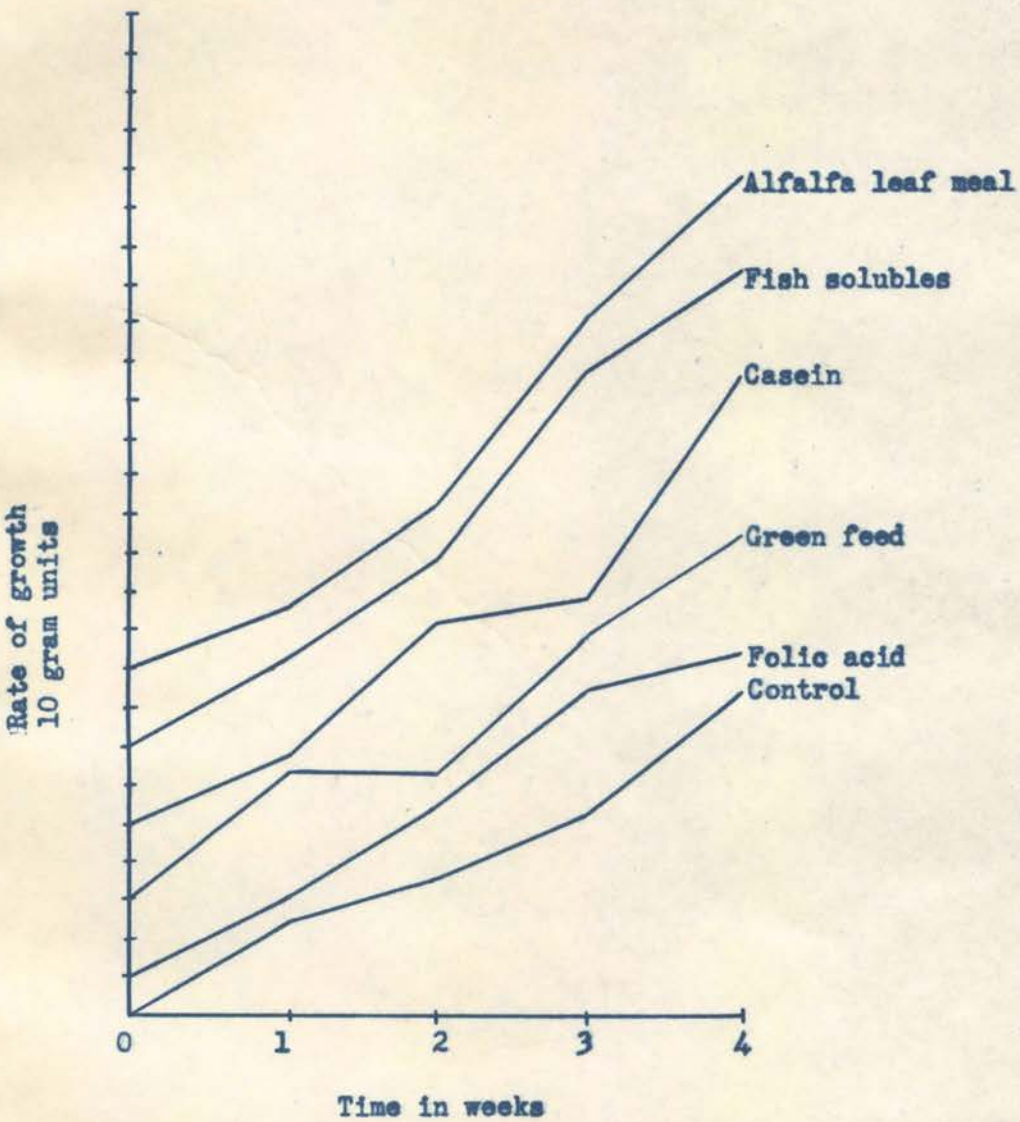
A second purpose of this experiment was to test various materials for the presence of an unidentified growth factor which might be the same as the growth substance found in fish solubles.

In order to do this, a mixed vegetable diet, supplemented with minerals and the principle B-complex vitamins, was used as the basal ration. It will be noted that after 4 weeks of growth, the average gain in weight for those chicks in lot 5, fed all the B-complex vitamins, was 193 gm.; those in lot 4, fed the ration supplemented with 5% alfalfa leaf meal, was 276 gm.; those in lot 6, fed the ration supplemented with 1.10 mg. of folic acid per 100 gm. of feed, was 223 gm.; lot 7, fed the rations supplemented with 5% casein was 243 gm.; lot 9, fed the ration supplemented with oat sprouts and gras ad lib. was 227 gm.; lot 8, fed the ration supplemented with 3% fish solubles was 290 gm.

The ration supplemented with alfalfa leaf meal gave better results than did any of the other supplements with the exception of fish solubles. The ration supplemented with 3% fish solubles gave significantly better results than did any of the other supplementations tried. The growth curves are shown in Figure 5.

A basal ration consisting of corn and soybean oil meal supplemented

Figure 5. Growth rates of chicks in trial 1 fed various supplements.



with minerals and B-complex vitamins was used in testing the effect of methionine and whey fermentation solubles on the growth of chicks. Di-methionine was added to the basal ration at the level of 1.54 gm. per 100 gm. of feed equivalent to and addition of 0.2%. Compared to the gain of chicks on the basal ration supplemented with B-complex vitamins, there was no significant difference in the gain of weight of chicks on the methionine supplemented ration. Whey fermentation solubles added at the level of 3%, resulted in a significant gain in weight over the basal ration. This gain was not nearly so great as the gain of the chicks on the ration supplemented with fish solubles.

A further comparison of supplements for the corn and soybean oil meal ration was studied in trial 4. For an unexplainable reason, the ration supplemented with all the B-complex vitamins except folic acid, produced a greater gain than did the basal ration supplemented with the B-complex vitamins.

A second trial using the same diets and under similar conditions, except for the use of a different breed of chicks, was run to check the results of trial 1. The results were essentially the same, except the average gains in weight of the chicks was slightly less. The relative positions of the lots on the variously supplemented rations were the same. Figures 6, 7 and 8 show the average gains in weights of the chicks for this trial.

A third trial was designed to study the effect of each of the principle B-complex vitamins on the growth of chicks. A basal ration of corn and soybean oil meal, supplemented with minerals was used. Each of the B-complex vitamins except thiamine, inositol, and para-amino benzoic acid was omitted singly to determine the effect on growth of chicks.

Figure 6. Growth rates of chicks in trial 2 on a mixed all-vegetable ration supplemented with vitamins and fish solubles.

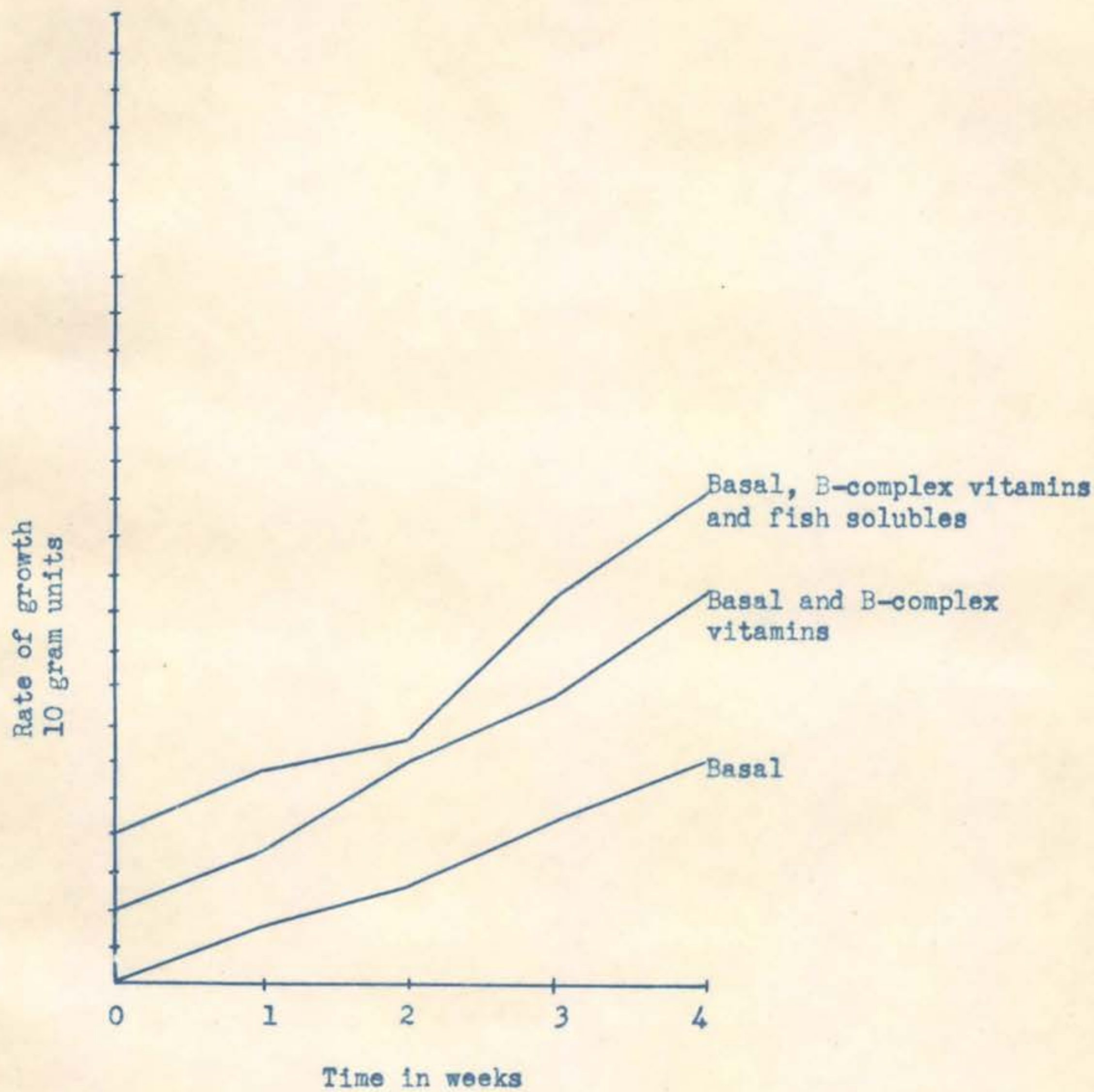


Figure 7. Growth rates of chicks in trial 2 on corn and soybean oil meal rations supplemented with vitamins and fish solubles.

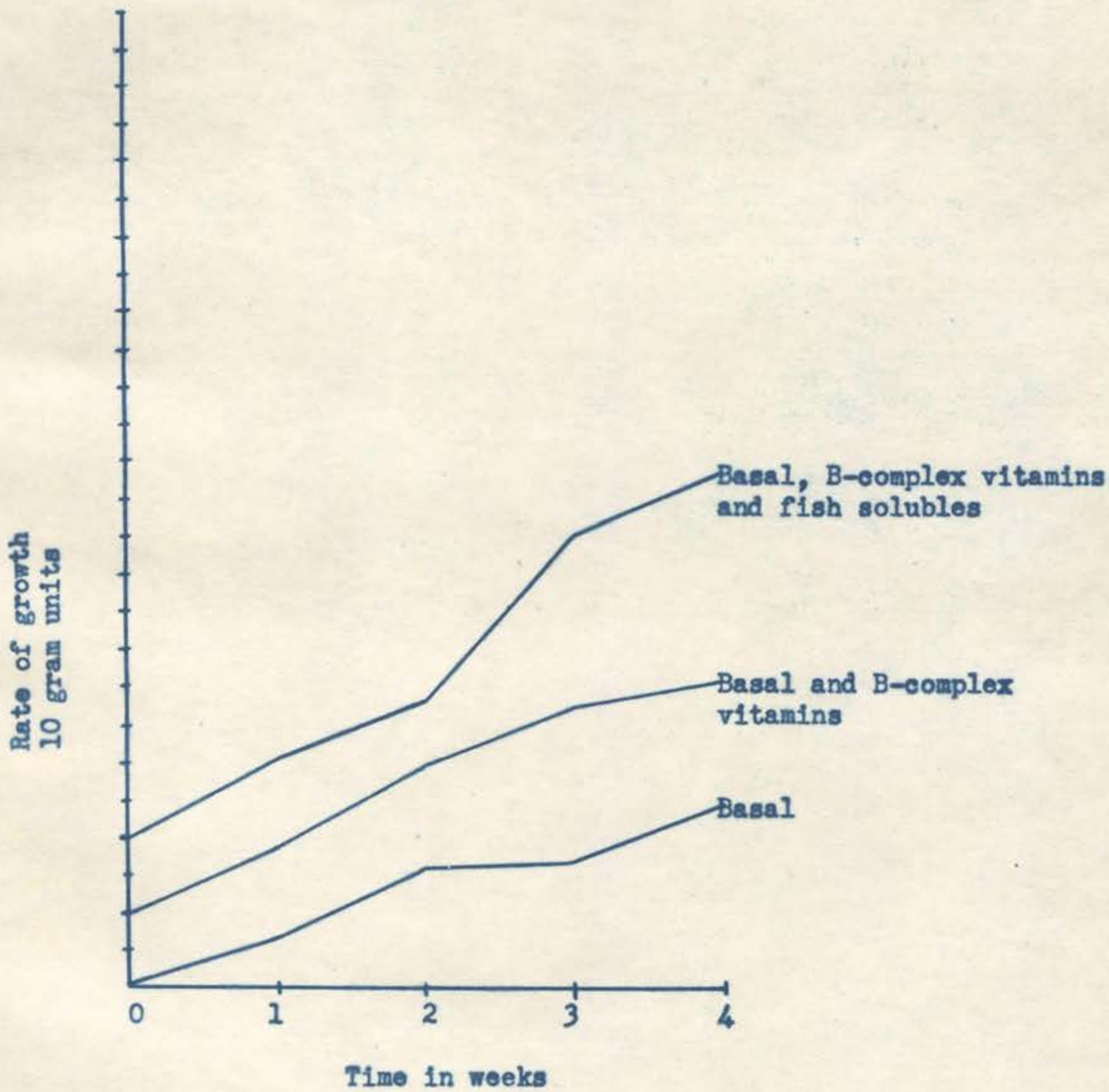
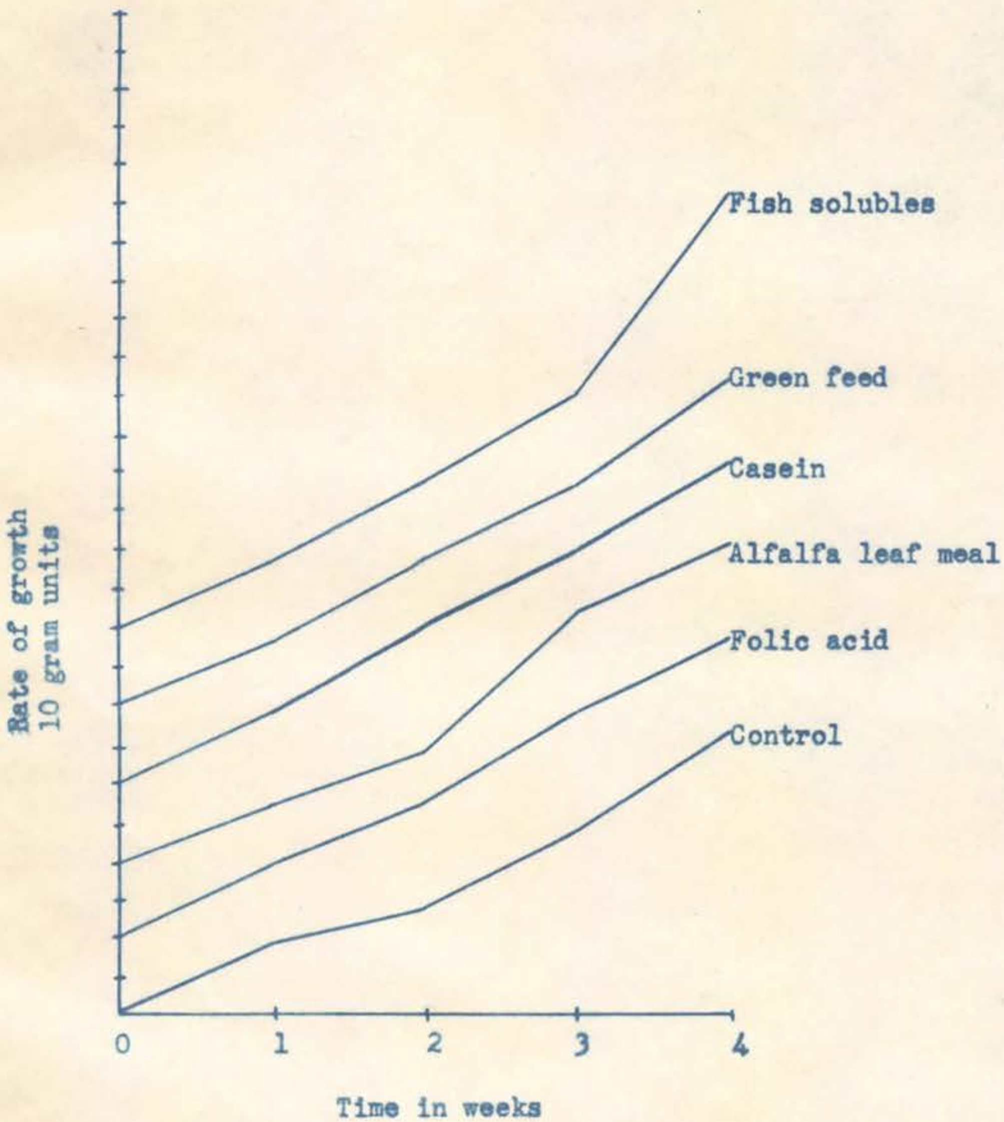


Figure 8. Growth rates of chicks in trial 2 with various supplements.



From table VII it will be noted that the average growth rate of the chicks in lot 34, fed the ration supplemented with all of the B-complex vitamins minus riboflavin, was greatly depressed and a typical riboflavin deficiency was evidenced as early as one week of age; lot 28, fed a ration supplemented with all of the B-complex vitamins and lots 23 and 33, fed a ration minus folic acid and choline respectively, showed a reduction in the growth rate which was evidenced particularly in the fourth week.

Lots 29, 30 and 31, fed a ration minus nicotinic acid, pyridoxine, and pantothenic acid respectively, did not show a depressed growth rate, their total average weight exceeded that of the lot receiving the ration supplemented with all of the B-complex vitamins; lot 27, fed the basal ration only grew slowly, but did not show signs of a riboflavin deficiency.

The significance of the reduced growth rates noted in lots 32 and 33 is questionable in view of a similar reduction in lot 28. The data seems to point to the fact that more significant results would have been obtained if this experiment could have been continued for at least two more weeks. This was not feasible because of the extreme heat.

One explanation of the fact that the chicks in lots 29, 30 and 33 showed an average gain better than the average for lot 28 may be that adequate amounts of these vitamins were present in the basal rations.

The fact that the chicks on the basal ration with no B-complex vitamins added grew slowly, but did not show any riboflavin deficiency is in conformity with the observation in man and other animals that a pronounced deficiency is more easily produced on a diet adequate in all respects except for a single vitamin.

Lot 35, fed a ration supplemented with 3% fish solubles and all the B-complex vitamins again showed greatly enhanced rates of growth, over all the other lots. The explanation for this seems to point to the fact that there must be some factor or factors over and above the known vitamins that is necessary for good chick growth.

A second set of experiments was conducted for the purpose of determining which fractions of fish solubles might contain the active growth factor.

Trial 4 was designed to test two concentrations of alcohol and fish soluble fractionations. The first a 50% concentration of alcohol and fish solubles was separated to include the following fractions: alcohol precipitate, norite filtrate, norite eluate, and norite absorbate.

Each of these factions was added to a corn and soybean oil meal basal ration, supplemented with minerals and the B-complex vitamins, in the equivalent amount of 5% fish solubles. The control lot was fed the basal ration supplemented with 3% fish solubles. The equivalent amount of 5% fish solubles used for addition of fractions to rations was thought to be necessary to compensate for loss in processing.

The norite filtrate, norite eluate, and norite absorbate fractions produced approximately the same growth rates for the first six weeks. The chicks fed the ration supplemented with fish solubles, gained approximately 25% more weight than did those fed either of the three norite fractions.

The alcohol precipitate fraction produced more gain than did the basal ration supplemented with fish solubles. This may have been due to a difference in the two lots of chicks or to the presence of a greater quantity of the growth factor in the fraction than in the supplemented basal ration.

A second concentration was separated which contained 75% alcohol and 25% fish solubles. The fractions from this concentration which were tested were: alcohol insoluble, alcohol soluble, and alcohol precipitate. These three fractions were used in the same manner as the fractions just previously discussed.

The gain in weights for the lots fed the alcohol precipitate and the alcohol soluble fractions were almost identical with the lot fed the basal ration supplemented with fish solubles. The gain for the lot fed the alcohol insoluble fraction was only 80% of that for the basal ration supplemented with fish solubles.

In trial 5, a concentration of 66 2/3% alcohol and 33 1/3% fish solubles was separated to yield the following fractions: alcohol insoluble, alcohol precipitate, alcohol soluble, norite filtrate, norite eluate, and norite absorbate.

Each of these fractions was added to the basal ration described in trial 4 in the amount equivalent to 3% fish solubles. The control lot was fed the basal ration supplemented with 3% fish solubles. No extra amount of the fractions was allowed to make up for the loss in processing.

The lot fed the alcohol insoluble fraction showed the greatest gain in growth, being approximately the same as the lot fed fish solubles. The lot fed the alcohol precipitate fraction showed a gain of almost as much as the lot fed the alcohol insoluble fraction. The alcohol soluble, the norite filtrate, and the norite eluate supplemented lots showed approximately the same amount of gain as the lot supplemented only with the B-complex vitamins, but significantly less than the alcohol precipitate fraction.

The lot supplemented with the norite absorbate fraction showed very poor growth, which might have been due to the absorption by the norite of other vitamins in the chick intestine.

Thus, in each of the three different concentrations of alcohol and fish solubles, the active portion of the fish solubles was found in a different fraction in each case. In trial 4, using a 50% alcohol concentration, the alcohol precipitate fraction showed the greatest growth stimulation. The alcohol soluble fractions treated with norite showed no significant gains. However, by using the 75% alcohol concentration of fish solubles, equal stimulation was shown by both the alcohol precipitate and the alcohol soluble fractions. The lot fed the alcohol insoluble fraction did not show a significant gain. In trial 5, using a concentration of 66 2/3% alcohol and 33 1/3% fish solubles, the alcohol insoluble fraction showed the greatest stimulation of growth in the chicks.

Incomplete separation of the fractions, or the difference in the concentrations of alcohol used may account for the results obtained.

SUMMARY

Chicks fed an all-vegetable ration supplemented with minerals and synthetic B-complex vitamins showed as great a gain in weight during 4 and 6 weeks periods as did chicks fed a practical poultry ration containing animal protein.

When the all vegetable ration was supplemented with B-complex vitamins and 3% fish solubles, growth rates of the chicks were greatly increased. Addition of folic acid, casein, green feed, alfalfa leaf meal, methionine or whey fermentation solubles to an all vegetable ration supplemented with B-complex vitamins did not stimulate growth of chicks as did the addition of fish solubles.

Chemical fractionation of fish solubles is described. The fractions were tested for the presence of the active growth factor in fish solubles. In a 50% and a 66% alcohol concentration of fish solubles, the active portion was found to be alcohol insoluble. In a 75% alcohol concentration of fish solubles, both the alcohol soluble and the alcohol insoluble fractions showed the same growth stimulating activity. The difference in the results may be due to incomplete separation of the alcohol fractions of fish solubles.

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