GROWTH FACTOR STUDIES WITH ALL VEGETABLE PROTEIN CHICK RATIONS

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INTRODUCTION

Research workers agree that an unidentified factor, or factors, is needed for the optimum growth, hatchability and reproduction in chickens in addition to those nutrients now known to be essential. Since there is a possibility that the same factor may be involved in growth, lactation, and reproduction with other farm animals as well, research has been conducted along several different lines. No chemical assay for the unidentified factor is known at the present time. Bio-assays using rats, chickens, and certain bacteria have been the only means of measuring the potency of known sources of this unidentified factor.

The amount of the unidentified factor required for optimum growth in the chick has not been fully established. It is recognized by nutritionists that feedstuffs of animal origin are usually good sources of the unidentified factor. However, processing techniques now employed by the manufacturers of many feedstuffs of animal origin destroy or preclude the factor. For this reason the addition of the factor to a poultry ration will often times give an increase in growth, reduce mortality and improve feed efficiency even though by all nutritional standards the ration is adequate and well balanced.

The purposes of this study are:

(1) To study the properties of the unidentified growth factor (s).

(2) To measure the growth responses obtained by feeding extracts of fish solubles.

(3) To determine the level of fish solubles in the ration which will produce optimum growth.

(4) To determine to what degree chick mortality is influenced by breeder and chick starter rations which are devoid of or contain very little animal protein factor.

(5) To study differences in feed utilization as influenced by the factor.

REVIEW OF LITERATURE

It has been a common belief for many years that a poultry ration to be adequate must contain a certain amount of animal protein. Since the value of animal protein in poultry rations was an accepted fact, very little research was conducted prior to 1941 to determine why animal proteins were essential.

Early observations by Kawakawi, Uchida and Nakamura (1927) showed that animal protein in the form of dried fish caused a more rapid growth in mice than did a diet containing plant proteins. Fronda (1927) found that the addition of fish meal to a ration not only stimulated growth in chickens, but also increased egg production and reduced mortality. Hayward and co-workers (1937) advised that 2 percent of dried skimmilk and meat scraps be used in addition to soybean oil meal in rations for poultry. These workers observed the benefits derived from feeding animal proteins, but the reason for its superior feeding value was not determined at that time.

During the years 1941-1946, plant protein was substituted for animal protein in poultry rations because the plant protein was more readily available and lower in cost. Research workers found that optimum growth was not obtained on the plant protein diets even though the ration contained all of the known nutrients essential for poultry. Through substitutions of this kind workers began investigations to learn why animal protein was superior to plant protein and what could be done to improve plant protein rations. For the assay work, most research workers used a corn-soybean meal basal ration.

Phillips, Carr, and Kennard (1920) fed several lots of chicks on diets which contained protein from soybean meal and meat scraps. They reported greater gains in weight from the ration containing only soybeans as the source of protein. Tomhave and Mumfaro (1933) reported poor growth in chicks fed ground raw soybeans. Ground raw soybeans when substituted for meat scraps in the ration increased mortality, retarded growth, decreased the efficiency of the ration, and produced uneven growth. Hayward, Steenbock and Bohstedt (1936) showed that rats required additional cystine in their ration when its protein was supplied by unheated soybean oil meal.

Hayward and co-workers (1937) found that soybean oil meal cooked at 98 degrees C. for 15 minutes resulted in an increased rate of growth in chicks and a greater efficiency of feed utilization over a ration containing raw soybean oil meal. In 1946, Kunitz, reported the presence of a protein in soybean oil meal which inhibits the proteolytic action of trypsin. Ham and Sandstedt (1944) were able to obtain satisfactory feeding results from soybean oil meal proteins when the proteolytic substance was removed.

Rubin and Bird (1947) suggested the possibility of a heat-stable growth inhibitor in soybean oil meal different from the heat-labile trypsin inhibitor reported by Kunitz. The addition of the growth factor found in cow manure helped to counteract the heat-stable inhibiting factor in the ration, Rubin and Bird (1947).

In addition to destroying the trypsin inhibitor, heating of soybean oil meal also makes available some of the amino acids, especially the sulphur-bearing ones, Almquist (1942) and Hayward, et al.(1936).

Almquist also found that heated soybean protein is deficient in methionine for the chick when fed at a 20 percent protein level. Since most plant protein diets are deficient in one or more of the essential amino acids, Almquist recommended the addition of proteins from animal sources. Clandinin, Gravens, Halpin, and Hart (1946) reported that some soybean oil meal rations were improved by the addition of choline, while others were not improved. They concluded that choline and methionine were not interchangeable in a soybean oil meal ration. Berry and co-workers (1946) showed that the feeding value of soybean oil meal rations could be increased by supplementing them with corn gluten meal. They suggested that this increase in value was due to the extra methionine present in the corn gluten meal.

Melnick, Oser, and Weiss (1946) reported that the rate of enzymatic digestion of proteins constituted a factor in nutrition. By the in vitro test, he discovered that proteins were affected at different rates by enzymic digestion. Since amino acids have to be available in adequate quantities and available simultaneously for proper utilization, he concluded that the protein hydrolysates should be superior in biological value to the original intact proteins. Woolley (1945) showed that there may be one or more essential peptides which the animal is unable to synthesize. He suggested that in addition to a proper balance of the essential amino acids, it may be necessary to insure certain peptide linkages in the diet for satisfactory utilization of amino acid nitrogen.

Almquist (1946) suggested that terms like "animal protein", "vegetable protein", and the like should be discarded. He pointed out that the important thing was an adequate supply of the essential amino

acids required by the animal regardless of the origin.

Hammond and Titus (1944) recommended the addition of vitamins and minerals to a soybean meal diet in order to obtain optimum growth.

Marvel et al.(1944) found that the addition of minerals and vitamins, including choline, to a corn-soybean oil meal ration produced growth in chicks equal to that obtained from the meat and bone scrap control ration. These same authors (1945) reported that a similar ration supplemented with 5 percent of distillers' dried solubles failed to supply enough riboflavin, pantothenic acid, and choline for adequate growth. However, a level of 10 percent of distillers' dried solubles did produce rapid growth.

Deobald and co-workers (1937) suggested that soybean oil meal may lack certain of the "B-Complex" vitamins. This suggestion was followed in work by Mishler and co-workers (1946). Their results showed that animal proteins could be omitted from a ration when the corn-soybean oil meal diet was supplemented with riboflavin, choline, nicotinic acid, minerals, and vitamins A and D. They suggested that the increased growth rate commonly believed to be caused by the proteins in animal products was actually due to the presence of certain vitamins. The results obtained by Emerson (1948) showed that when an all-vegetable ration was supplemented with certain B-Complex vitamins, the growth rate of chicks was increased. Even greater growth was obtained with the addition of 3 percent of fish solubles. The addition of folic acid, casein, green feed, alfalfa leaf meal, methionine and whey fermentation solubles to the basal which was also supplemented with B-Complex vitamins did not bring about the same rate of growth

as was obtained with fish solubles.

Cravens, McGibbon, and Halpin (1945) indicated that protein quality was not the chief factor responsible for the increase in growth obtained from animal products. These authors observed an improvement in the growth of chickens when condensed fish press water or ground fish viscera was added to a diet composed of yellow corn, wheat by-products, meat scraps, soybean oil meal, minerals, fish oil, and riboflavin. This indicated that the fish press water or viscera contained a factor not present in these meat scraps, or not in as high a quantity as was found in the other two sources mentioned. Their findings were further substantiated by Carver and Evans (1943). Norris and Heuser (1943) were able to obtain satisfactory growths in chicks fed a ration containing soybean oil meal as the principal source of protein. However, they advised the addition of 10 percent of protein of animal origin as a safety margin. They reported that fish meal was more effective as a supplement to soybean oil meal than was meat scraps. Heuser, Norris, and McGinnis (1946) observed that livability was improved by the addition of fish meal to an all-vegetable protein ration.

Patton and co-workers (1946) stated:

> "that the nutritional effects of these animal supplements are due either to still unidentified substances or factors, vitamin-like in character, or to certain little understood relationships or interactions involving known nutrient materials." "Plant protein diets appear to be lacking in an unknown factor which is present in sardine fish meal", they concluded.

McGinnis, Stevens, and Groves (1947) found that an unidentified factor was extracted from liver by ethanol. It was necessary for growth and livability in chicks fed a diet composed of cereal grains.

The factor does not appear to be influenced by the type of protein in the diet. Methionine gave a growth response in addition to that obtained by the factor; although, it did not replace the factor. They reported that the unidentified factor was destroyed by oxidation, was soluble in water at a pH of 5.0, 4.0, 3.0, and 2.0, and that it was soluble in acetone. It was dialyzable through a cellophane membrane.

Hill (1948) suggested that two unidentified nutrients are concerned in the growth of the chick. One is found in whey, soybean oil meal, and fish solubles, while the other one is found in meat scraps. However, Christiansen and co-workers (1940) reported that meat scraps were not valuable in combination with soybean oil meal. They found a great variation in the value of different samples of meat scraps.

McGinnis and Carver (1947) reported that the addition of fish meal and an alcohol-soluble liver fraction to a corn and soybean oil meal diet increased growth and reduced mortality. The chick's requirements for the factor, or factors found in fish meal did not appear to be dependent on the presence of corn or soybean oil meal in the chick's diet. Mishler and co-workers (1947) found that fish solubles were an excellent supplement in corn and soybean oil meal rations for young chickens. They observed that male chicks gave a greater response to fish solubles than did female chicks. Berry and co-workers (1945) showed that 2 percent of fish meal, or 2 percent of fish liver, was a good supplement to a corn and soybean oil meal ration. Similar recommendations were made by Heuser and Norris (1944).

The results of experiments by Whitson, et al. (1945) showed that the addition of 3 percent of sardine meal or 8 percent dried cow manure

supplied the factor, or factors, needed in the soybean oil meal ration. Mussehl (1931) reported that fish meal was superior to casein in chick rations.

Robblee and co-workers (1947) supplemented a basal ration composed of natural feedstuffs with fish solubles. Results of this supplementation showed that a definite increase in growth was obtained with chicks. The same response was obtained on a purified diet. Hill and Van Poucke (1947) reported that the unidentified factor was also required by chicks on a purified diet. A supplement of fish solubles, milk products, and gelatin increased growth in chicks when added to the purified diet.

Johnson, Carrick, Roberts, and Hauge (1942) showed experimentally that there is a factor present in casein and dried liver meal which is essential for the growth of the chick. It is distinct from vitamin A, thiamin, riboflavin, nicotinic acid, pantothenic acid, para-aminobenzoic acid, choline and pyridoxine. The factor is soluble in ether and ethanol and is thermostable.

Berry, Carrick, Roberts and Hauge (1943) found a growth promoting factor, or factors, in whey which was not present in either corn or soybean oil meal. Casein was another source for the factor. Novak, Hauge, and Carrick (1947) reported an unidentified growth factor, or factors, necessary for chicks in distillers' dried solubles. The factor, or factors, is distinct from vitamin A, vitamin D, thiamin, riboflavin, pyridoxine, pantothenic acid, niacin, choline, biotin, folic acid, para-aminobenzoic acid, 2-methylnaphthoquinone and inositol. Schumacher, Heuser, and Norris (1940) reported two growth factors

required by the chick. Both factors are found in dried brewers' yeast. The factors are extracted from yeast with 0.24 N HCl solution and are separated by alcohol precipitation upon adjustment of the pH. One of the factors, (Factor R) is soluble in acid alcohol, while the other, (Factor S) is precipitated in this solution.

Zucker and Zucker (1948) found an unidentified factor which they named "Zoopherin." It was present in 1:20 liver powder, fish solubles, and crude casein. They found the factor to be soluble in water, dilute acid and alkali, and dilute alcohol, moderately soluble in 95 percent alcohol and insoluble in petroleum ether and ethyl ether. It was stable to heat, light and air.

Recent work showed that certain bacteria require growth factors which are also essential for the chick. Mills and co-workers (1942) found that the liver fractions which were rich in the norit eluate factor also contained the L. casei factor necessary for feather development and hemoglobin formation as well as for growth. In feeding chicks on a purified diet, Briggs et al (1943), demonstrated the existence of two necessary dietary factors in liver and other materials distinct from folic acid. These authors named the factors Vitamin B_{10} which they found for feather development, and Vitamin B_{11} which was essential for growth. Both factors were soluble in water, alcohol and ammonia and precipitated by ethyl alcohol.

Hogan and Parrott (1940) reported finding in liver a factor essential for poultry which they called Vitamin B_c. It served to prevent anemia from occuring in chicks. It was not identified with any of the known vitamins.

In 1944, Hutchings and others isolated a crystalline compound which is active for L. casei and S. lactis R., and is also active in the nutrition of the chick. Work by Hutchings, Oleson, and Stokstad (1946) showed that the fermentation Lactobacillus casei factor and the synthetic liver Lactobacillus casei factor were active in promoting growth and hemoglobin formation in the chick.

Scott and co-workers (1945) showed that the addition of the L. casei factor to a basal ration would prevent the development of a certain type of anemia in chicks.

Stephenson and co-workers (1948) reported finding a factor in soil which was necessary for chick growth. An increase in growth occurred by the addition of soil to a soybean oil meal ration. A liver fraction was found more effective for chick growth than was the soil factor. McGinnis et al (1948) suggested that the growth promoting factor found in soil might be due to the presence of Bacillus subtillis organisms which have been shown to contain a growth promoting factor.

In experimenting with built-up floor litter, Kennard and Chamberlin (1948) pointed out that built-up floor litter served as a potent source of the nutritional factors, including the unidentified animal protein or vitamin factor(s). Rubin, Bird, and Rothchild (1946) found a chick growth factor in the droppings of hens. They believed the growth factor was synthesized since it was not in the hen's diet. McGinnis, Stevens, and Groves (1947a) were able to stimulate synthesis of an unidentified factor, or factors, in hen feces by incubating the feces for seventy-two hours at 30 degrees C. Feces which were frozen following collections contained little or none of the factor. They concluded

from these results that the synthesis of the unidentified growth factor, or factors, in hen feces takes place, at least in most part, after voiding of the feces and not to any extent in the digestive tract.

Rubin and Bird (1946a) reported a chick growth factor in cow manure. The factor in cow manure, when added to an all-plant protein diet, gave optimum growth. These authors found that the factor was not destroyed when the manure, from which it was obtained, was sun dried or oven dried. Hammond (1944) reported that dried cow manure was a good source of riboflavin. Sardine fish meal did not supplement dried cow manure in supporting growth and feed efficiency. Bird, Rubin and Groschke (1948) reported that the growth factor in cow manure was soluble in water at a pH of 3.0, if the proteins present were previously removed by digestion. The factor was soluble in 80 percent acetone and could be extracted slightly by neutral ethanol. It was stable when autoclaved 2 hours at a neutral pH, but readily destroyed by autoclaving 1 hour with 2 N acid. Rubin and Bird (1946) found the factor soluble in 50 and 95 percent alcohol, but insoluble in chloroform and ether. Emerson (1948) reported the active growth factor soluble in 75 percent alcohol and insoluble in 95 percent alcohol. Stokstad and Manning (1938) reported a factor in alfalfa, middlings, wheat bran, and yeast which is required by chicks. They found the factor insoluble in ether, acetone and isopropanol, but soluble in water and in mixtures of water and methanol. Autoclaving yeast did not destroy the growth factor while autoclaving alfalfa did destroy it.

Riley, Hammond (1942), and Rubin and Bird (1947), reported an androgenic substance in the feces of cows in various stages of

gestation and of unbred heifers. The development of the testes and ovaries of chicks was retarded when chicks were fed materials containing the active factor. Feces from mature bulls did not effect either comb growth or gonadal development. Hammond (1942), however, suggested that cow manure would not cause deleterious effect on the growth of chicks if it is added to a complete and balanced diet.

The chick growth factor found in cow manure is transmitted by the hen through the egg to the chick, Rubin and Bird (1946). The factor is not identical with the L. casei factor (from liver, yeast or fermentation residues), factors U, R, S,, Vitamins B_{10} or B_{11}, synthetic folic acid (Lederle) or Pyrotin lactone. In 1947 these same workers found that when cow manure was fed most of the factor from cow manure was stored in the yolk of the egg. The factor was present in the acetone insoluble fraction of the egg yolk. Robblee et al, (1948) reported an unidentified factor in condensed fish solubles which increased growth in chicks on a diet adequate in the known growth nutrients. The addition of three percent of fish solubles to a purified diet resulted in a different growth response than was found by the addition of three percent fish solubles to natural rations. They observed that the degree of growth response obtained in chicks is influenced by the diet fed the hens. Similar reports were made by Bethke, Pensack, and Kennard (1947). McGinnis, Stevens and Groves (1947) also reported that the need for the factor is influenced by the type of diet fed the breeders. When high levels of the factor were fed the breeders, a need for the factor in the chick's diet could not be shown.

Intensive work by Bird, Rubin and Groschke (1947) shows that chickens may vary widely with respect to their need for the dietary

factor. This variation might be caused by the amount of stored factor present or some characteristic which influences their ability to withstand the deficiency. They found that there was a great variation in the hatchability of hens fed a diet containing no animal protein for 11 lunar months. Hatchability records of 183 hens on this diet revealed that 44 percent of the hens showed hatchability figures between 0 and 70 percent, 37 percent showed from 70 to 80 percent hatchability, and 19 percent showed from 85 to 100 percent hatchability. They believed that the variability among the hens was an inborn characteristic. Whether the variation of the progeny was congenital or hereditary was not stated.

Most research workers found that three percent of fish solubles would produce optimum growth and that no benefits were derived from the feeding of higher levels. However, Lassen and Bacon (1946) reported increased growth rates in chicks fed fish solubles at a level as high as 12 to 13 percent of the ration without any serious effects.

In addition to stimulating growth, fish solubles and other meat products have proven beneficial in egg production and hatchability. Deobald, Halpin and Holmes (1937) were able to increase hatchability of the fertile eggs by the addition of as little as 2 percent meat scraps and 2 percent dried milk. Whitson, Titus, and Bird (1946) reported that diets consisting largely of corn and soybean meal with vitamins and minerals caused a decrease in hatchability. Hatchability was increased and seasonal variation was eliminated by the addition of the unidentified dietary factor found in cow manure to a corn-soybean oil meal ration, Groschke, Rubin, and Bird (1947). Nestler, et al. (1936) reported that hatchability was greatly increased by the addition

of dried pork liver, green grass and meat products to an all grain ration. They found that the factor was not present to any extent in whey.

Christiansen, Halpin, and Hart (1940) observed that the addition of protein supplements of crude casein to a soybean oil meal diet did not eliminate the winter slump in hatchability. They were able to eliminate the winter slump, however, by the addition of manganese or riboflavin. Wilgus and Gassner (1941) reported poor hatchability and high mortality of the chicks hatched from hens on a ration which was adequate in riboflavin and manganese, but did not contain any meat scraps. They pointed out that the poor reproduction might have been caused by the goitrogenic effect of the soybeans. Heuser, Norris, Lucas, and Combs (1946) were able to increase hatchability in eggs from hens on a soybean meal ration by the addition of the known B vitamins. They suggested that the unknown factor was synthesized by the hens or by microorganisms. Byerly, Titus, and Ellis (1933) using diets consisting of cereal grains, were able to increase hatchability by letting the hens have access to the range, and by the addition of milk and animal proteins to the diet. Parkhurst and Kuzmeski (1944) reported that excellent hatchability was obtained on vegetable diets containing 20 percent of distillers' dried grains with as little as 1.25 percent of flame dried redfish meal. Very poor hatchability was obtained from all groups receiving diets not containing some animal or marine protein concentrate. Parkhurst, Fellers, and Kuzmeski (1945) found that fish meal was a more valuable supplement to distillers' by-products than meat scraps for hatchability and increased efficiency of feed utilization. These authors observed that corn distillers' by-products had little, if any effect on egg quality.

Byerly, Titus, and Ellis (1933) reported that diets containing proteins from vegetable sources increased the incidence of chondrodystrophy in the embryos of hens likely to produce such embryos. Embryos in eggs from hens on such diets had a high second week mortality. McGinnis and Carver (1947) reported high mortality in chicks produced from hens fed soybean oil meal plus 1.7 percent fish meal. A supplement of 4.6 percent of fish solubles to the soybean oil meal, however, permitted storage in the egg sufficient to meet the requirement for maximum growth and livability.

Observations were made by Bethke and co-workers (1946) on the influence of nutrition to production and hatchability. They stated that soybean oil meal diet may be satisfactory for egg production, but is deficient in a factor, or factors, essential for good hatchability. The growth factor was present in meat scraps, menhaden fish meal, and dried skimmed milk. Further work by these authors (1946, 1947) showed the factor to be present in meat scraps, dried pork liver, liver extract and condensed fish solubles. These supplements increased the hatchability of eggs when added to the all-vegetable breeder ration. Choline Chloride and d.l. methionine, either alone or in combination, did not improve hatchability. The factor was found in sardine fish meal and was not pantothenic acid, niacin, pyridoxine, folic acid, L. casei factor, or biotin.

Gillis, Heuser, and Norris (1942) reported that a factor, or factors, present in liver extract had little or no effect on hatchability except in the presence of pantothenic acid. The addition of pantothenic acid alone to the basal diet increased hatchability from zero to 10.30 percent. When it was added to the basal diet along with

the factor, or factors, in liver extract, hatchability was increased to 50-60 percent. These authors concluded that pantothenic acid and the factor in liver were essential for hatchability.

McGinnis, Heuser, and Norris (1944) noted that the factor was not present in dried brewers' yeast and was different from Choline. Hatchability was increased by the addition of 3 percent of meat scraps or 0.2 percent of liver paste.

Petersen, Wiese, and Lampman (1948) reported better hatchability from hens which had access to an open pen, than was obtained from hens receiving the same vegetable diet in cages. Evidently the hens in open pens were obtaining some essential factor by caprophagy. The addition of meat meal and herring fish meal improved the hatchability of the eggs from hens in open pens. This was in agreement with earlier work reported by Rubin, Bird, and Rothchild (1946), Kennard and Chemberlin (1948). Bird and Marvel (1943) were able to increase hatchability by adding 10 percent of dried feces to the ration. Since one would expect less bacterial synthesis in feces during the winter than in the summer, they concluded that the failure of hens to receive any benefit from the feces during the winter would possibly be responsible for the seasonal variation. Whitson, Titus, and Bird (1946) found that the inclusion of 8 percent of dried cow manure to a soybean meal diet had no effect on egg production, but it did increase hatchability and largely eliminated the seasonal variation in hatchability which was present in hens on the basal diet. Similar results were shown by Bird, et al.(1946). These authors suggested that the same deficiency in a diet which causes low hatchability, could also cause low viability of the chicks.

It is believed by some workers that chick age has an influence on the chick's requirement for the unidentified factor. Heuser and Norris (1944) reported that in order to obtain optimum growth and efficient feed utilization in chicks up to 8 weeks of age, a soybean oil meal protein ration should contain a minimum of 2 to 3 percent animal protein, such as, fish meal, meat scrap, or dried skimmilk. After two months of age, a smaller amount of animal protein will produce satisfactory results. Wilgus and Zander (1945) showed in one experiment that egg production was satisfactory from hens on a soybean oil meal ration. In another experiment, however, the onset of production was retarded when birds were reared and maintained into the laying period on the soybean oil meal ration. These authors advised the addition of 5 percent of meat and bone scraps to the mash in order to provide satisfactory production and reproduction.

Brant and Carver (1947) ran an experiment on chickens from one day of age through the first laying year in order to see what effect soybean oil meal, as a protein supplement, would have on growth and reproduction. They found that soybean oil meal as the sole source of protein failed to give optimum growth up to 8 weeks of age; however, results obtained from 8 to 20 weeks indicated that growth and development during the laying period of the same hens were comparable to the results obtained from hens whose diets contained soybean oil meal in combination with fish meal, meat scrap, and fish meal plus cottonseed meal during the first 8 weeks of age. Bird and co-workers showed that the addition of 4 percent of fish meal to the diet during the first 8 weeks of the growing period did not improve the growth rate, age of sexual maturity, or subsequent egg production in hens any more than it did

from hens which did not receive the fish meal during the first 8 weeks.

Some workers believe that the unidentified factor necessary for growth, reproduction and livability in the chick is also associated with the factor necessary for reproduction and lactation in animals. Ross, Phillips, and Bohstedt (1942) found that a diet of corn-soybean meal was not adequate for lactation in rats or for the growth of the fetus. Similar findings were reported by Cunha (1944). Spitzer and Phillips (1946) noted that plant rations failed to support normal reproduction and lactation in rats. More than 35 percent of the females fed the basal ration were completely sterile. Reproduction and lactation was improved by the supplementation of additional alfalfa meal, 1:20 liver powder, a combination of casein (crude or acid-washed) plus Choline, or fish meal. Krider, et al, (1946) observed that condensed fish solubles and rye pasture contained a factor, or factors, which was transferred through the sows milk to the nursing pigs.

Cary and co-workers (1946) found that rats from mothers fed on a deficient diet grew slowly on a diet adequate in all known nutrients. The addition of a liver extract gave normal growth. The factor (x) was water-soluble, dialyzable and precipitated with ammoniom sulfate. Bowland, Ensminger and Cunha (1948) found that a purified ration containing six of the B-Complex vitamins, the alcohol-soluble liver fraction, folic acid, biotin, inositol, para-aminobenzoic acid, and 15 percent of dehydrated alfalfa failed to give optimum growth in rats. They concluded that rats require one or more unknown factors for growth in addition to the ten known B-complex vitamins.

The unknown factor(s) obtained from the liver fraction, and essential for the chick, gave no supplemental effect to the rat. These authors suggested that a different factor(s) was concerned in the growth of the rat or else the factor(s) differs in its availability to the chick and rat.

Zucker and Zucker (1948) found that rats were carried through pregnancy and lactation on a well fortified plant ration consisting of cottonseed meal. They observed from the weight of the mother and offspring that lactation was normal. When soybean meal was substituted for the cottonseed meal, lactation was depressed. If females were maintained on the cottonseed meal diet for many months and then bred, there was high mortality and poor pre-weaning growth of the offspring.

Reports from Merck's Laboratories (Rickes et al. 1948) indicated that Vitamin B_{12} might be the long sought for growth factor. Even though experimental work pertaining to this vitamin has been done on human subjects, there are reasons to believe that Vitamin B_{12} could be very closely associated, if not, identical with the factor, or factors, many workers have encountered.

Vitamin B_{12}, a crystalline compound in the form of red crystals, was obtained from liver by Rickes et al.(1948). Experiments carried on by Rickes et al.(1948) and West (1948) showed that the substance is active in controlling the hematological manifestations of Addisonion pernicious anemia.

Shorb (1947) in an attempt to find a microorganism that might require a rat growth factor found in liver extracts, certain caseins

and in some foodstuffs, found that Lactobacillus lactis Dorner required the presence of two unidentified factors for growth in an amino acid basal medium containing all the synthetic B vitamins. One factor, present in clarified tomato juice (TJ) was also found in low amounts in casein and in many other substances, while the second heat-stable factor (LLD) was found in highest concentrations in the liver extracts active for rat growth, but not in casein or casein hydrolysates. Since the LLD factor was found in liver extracts and its concentration was similar to the factor used in the treatment of pernicious anemia, it was suggested that the LLD factor might be the therapeutically active principle in these extracts.

West (1948) showed hematological response resulted when four patients who had pernicious anemia were injected with impure amorphous concentrates containing 20,000 - 40,000 LLD units. A rise in reticulocytes, red blood cell count, and hemoglobin indicated that the treated patients were recovering from the anemia. The rise in white cell and platelet count had risen from 120,000 to 340,000. Results obtained by the administration of the TJ factor to anemic patients indicated that it had no function in the treatment of pernicious anemia.

Dakin, Ungley and West (1936) confirmed their earlier reports that hematopoietic substances in liver are associated with a peptide, possessing many properties of albumase. This might have been associated in part to the now known Vitamin B_{12}.

Nichols, Robblee, Cravens, Elvehjem (1947) found that concentrated pernicious anemia preparations were highly active in promoting growth in the chick. It was found more active than crude liver preparations

and showed greater activity when injected than when given orally. Five-hundredths, or 1 U.S.P. unit per day, per chick, gave a maximum growth response. No difference in hemoglobin levels were found in any of the groups of chicks tested. Results showed that it is soluble in 70 percent alcohol and precipitated with 95 percent alcohol.

Rubin and Bird (1946a, 1946) showed that the growth factor present in cow manure was likewise soluble in water, 50 percent ethyl alcohol, and 95 percent ethyl alcohol. Growth responses from test indicated that the growth factor may be identical with the crystalline Vitamin B_{12}.

Rickes, et al. (1948), and Ansbacher (1948) suggested that the LLD factor was identical with Vitamin B_{12}. These authors found the LLD factor in fairly high amounts in a papain digest of the acid precipitate of cow manure, fish meal, pancreatin, papain, egg white, and in lower amounts in alcoholic extract of whey, soybean oil meal, gelatin, zein, and mylose p. enzyme. The TJ factor activity as reported by Shorb (1948) was also found in most of the same materials. The distribution of the LLD and TJ factor activities in these materials suggest that they may be involved in chicken nutrition.

The growth-promoting effect of crystalline Vitamin B_{12} was compared with that of a liver concentrate used as an arbitrary standard for the LLD factor assay. The standard was assigned a potency of 1,000 units/mg. On this basis, the potency of Vitamin B_{12} was found to be about 11,000,000 units/mg when a 23 hour growth period was employed and about 17,000,000 units/mg with a 42 hour growth period, (Shorb, 1948).

Rickes, Brink, Koniuszy, Wood, and Folkers, (1948) isolated a red crystalline compound from Streptomyces griseus, a microorganism which Shorb had reported as showing Lactobacillus lactis activity. The vitamin B_{12} crystals were compared with those isolated from Streptomyces griseus. When heated on the micro-stage, the crystals from S. Griseus lost their red color at about 212° and did not melt up to 320°. Crystalline B_{12} similarly darkened to black at 210-220° and did not melt below 300°. The crystals, after drying, were found to have refractive indices of 1.619 (alpha), 1.649 (beta), and 1.659 (gamma), which are in agreement with indices of 1.616 (alpha), 1.652 (beta), and 1.664 (gamma) for Vitamin B_{12}. Emission spectrographic analysis of the crystals revealed the presence of cobalt and phosphorus, as it did for crystalline B_{12}, (Rickes et al, 1948). Solubility tests showed that the crystals and crystalline B_{12} had approximately the same solubility in 80 percent acetone. They showed optimal "animal protein factor" activity for the chick at a level of 30 micrograms per kilo of diet, which was comparable with that found by Ott, et al.(1948) for Vitamin B_{12}. As little as 6 gamma per kilo of diet did stimulate growth. West (1948) tested these crystals and found that the clinical response in pernicious anemia parallels that shown by Vitamin B_{12}. These comparative data are evidence that the crystals from the microbiological source and Vitamin B_{12} are identical.

Rickes, Brink, Koniuszy, Wood and Folkers, (1948) stated that Vitamin B_{12} appears to be a cobalt coordination complex which, having 6 groups about the cobalt atom, could involve one or more organic moieties. The red color of B_{12} appears to be at least in part associated with its cobalt complex character. The presence of cobalt in Vitamin B_{12} reflects

significantly upon many biological studies which have shown that cobalt is an essential trace element in nutrition, and perhaps upon suggestions concerning cobalt as a trace contaminant in iron therapy of anemias.

Cobaltous ion (1 microgram/ml) was without activity for L. lactis as contrasted with the high potency of B_{12}, (0.000013 micrograms/ml, half maximal growth).

Spectrographic examination of B_{12} also showed the presence of phosphorus. Although nitrogen was found to be present, tests for sulphur were negative.

Vitamin B_{12} in 0.015 N Sodium hydroxide solution (0.2 micrograms/ml) was inactivated (microbiological assay) at room temperature as follows: 20% (0.67 hr), 45% (6 Hrs), 90% (23 hrs), and 95% (95 hrs); it was inactivated in 0.01 N. hydrochloric acid solution (10 micrograms/ml) as follows: 18% (3 hrs), 75% (23 hrs), and 89% (95 hrs).

Smith (1948) was able to detect cobalt in crystals by the borax bead test. Colorimetric estimation with nitroso-B-Naphthol showed the presence of 4.0 percent of cobalt crystals dried in vacuo at 56° C. If each molecule contains one atom of cobalt, the molecular weight of the compound is about 1,500. The same author found that the molecule contains three atoms of phosphorus.

METHOD AND PROCEDURE

A short assay method as recommended by Bird[1] was used in this study. The assay procedure is outlined below.

Day old chicks were wing banded and placed in an Oakes gas heated battery brooder. In order to deplete the chicks of the stored factor(s) the basal ration (no. 1) was fed for a two week pre-assay period. Water and mash were supplied ad libitum.

Since most workers have used corn-soybean rations for their assay work, it was thought advisable to use a similar ration in order to have a basis for comparing results.

Basal Ration No. 1 for Chicks

Ground Yellow Corn	50.0
Alfalfa Leaf Meal	5.0
Soybean Oil Meal	38.2
Distillers Solubles	2.5
Steam Bone Meal	2.1
Ca Co_3	0.9
Salt	1.0
Vitamin A and D Feeding Oil (3000 A, 400 D) .4 oz. Riboflavin	0.3
	100.0

Analysis[2] of Basal No. 1:

Water	Ash	Protein	Fat	Fiber	N.F.E.	Ca	P
7.82	8.06	22.00	2.07	3.95	56.10	1.45	.612

[1]Through conversation.

[2]Analysis made through courtesy of Dr. V. G. Heller, Agricultural Chem. Research Department.

Calculated Amino Acid Composition of Basal Ration No. 1:

Arginine	Lysine	Methionine	Cystine	Tryptophane	Glycine
1.519%	1.388%	0.523%	0.414%	0.293%	3.559%

At the end of the second week the chicks were weighed. The weight of the chicks was determined and an upper and a lower weight limit decided upon. This was usually plus or minus 20 grams. Chicks within these limits were selected at random and distributed into the various assay lots. These lots were housed in a warm-shaft electrically heated brooder. From 11 to 14 chicks comprised each lot.

Materials to be assayed were added to the basal ration and fed during the third week to the various assay lots. The rations containing the different supplements to be tested were randomly assigned among the several lots of chicks.

All experiments were terminated at the end of the third week. Each chick was weighed and the average weight for each lot found. Sex was determined and the average weights weighed in proportion to the number of males and females. The average increase in weight for each lot during the third week was the criteria used to measure the potency of the supplement tested. A record of mortality was kept during the first two weeks of each Experiment. Feed consumption records were kept during the third week only. This method, according to Bird and others, will satisfactorily measure the growth response obtained from any supplement added to the basal ration.

Fish solubles[3] in all cases were fed at a level of 3 percent. Extracts and precipitates were fed at an equivalent level, except as

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[3]Fish solubles furnished by the courtesy of Borden and Company, and James H. Seley and Company.

shown otherwise under each Experiment. The liquid fraction in each experiment was added to the feed and the excess moisture eliminated by drying the mash in a dryer at approximately 100 degrees F. The fish solubles used in Experiments 1, 2 and 3 were from a different source than that used in Experiments 4, 5 and 6. Feeding trials in Experiment 4 showed that the feeding value of the fish solubles from the two different sources was comparable.

EXPERIMENT 1

The chicks used in Experiment 1 were from Single Comb Rhode Island Red breeder hens which had been on an all-vegetable protein ration (Basal No. 2) for at least two months prior to the time eggs were saved for hatching. Rhode Island Red cocks were rotated at three week intervals in the breeding pens.

Basal Ration No. 2 for Breeder Hens	
Ground Yellow Corn	57.0
Alfalfa Leaf Meal	5.0
Soybean Oil Meal	30.0
Steamed Bone Meal	4.2
Ca Co_3	2.3
Distillers Solubles (Seagrams)	0.5
Salt	0.7
Vitamin A and D Feeding Oil .4 oz. riboflavin	0.3
	100.0

Extracts of fish solubles were prepared using water, 10 percent methanol, and 20 percent methanol. Two parts of fish solubles and one part of distilled water or alcohol solution were mixed in a Waring Blender for five minutes. The mixture was then centrifuged in an angle head centrifuge. Three distinct layers separated out. These were a liquid layer, a fat-like layer, and a solid clay-like residue layer. Each layer was carefully separated from the others by decanting and scrapping with a spatula. This extraction procedure was repeated six times. The soluble fraction was a clear, brownish color liquid. The different fractions were stored in a refrigerator with a temperature range of 30-45 degrees F. until they were added to the ration.

Feeding tests were made using the three fractions from each of the solvents. The average gains for each lot during the third week, and the pounds of feed consumed per pound of gain, are shown in Table 1, Fig. 1, Analyses[4] of the fish soluble fractions used are given in table 1 a.

From the results obtained, it is evident that the water soluble fraction of the fish solubles contained the "growth promoting" factor.

Mortality on 262 chicks during the two week pre-assay depletion period was 27.5 percent.

Table 1

Lots	Supplements	Ave. Gain 3rd Week	Lbs. Feed/ Lb. Gain
1	Regular chick starter (Mash used by Poultry Dept.)	55.8 gms.	3.57
2	Basal	31.9	4.18
3	Basal + 3 percent fish solubles	75.8	2.29
4	Basal + water extract of fish solubles	70.5	2.15
5	Basal + fat from fish solubles	31.6	5.12
6	Basal + residue from fish solubles	32.4	4.54
7	Basal + 10 percent methanol extract	56.1	2.86
8	Basal + fat from 10 percent methanol ext.	36.4	3.16
9	Basal + residue from 10 percent methanol extract	33.5	4.09
10	Basal + 20 percent methanol extract	62.0	2.74
11	Basal + fat from 20 percent methanol ext.	48.4	2.86
12	Basal + residue from 20 percent methanol extract	33.7	3.82

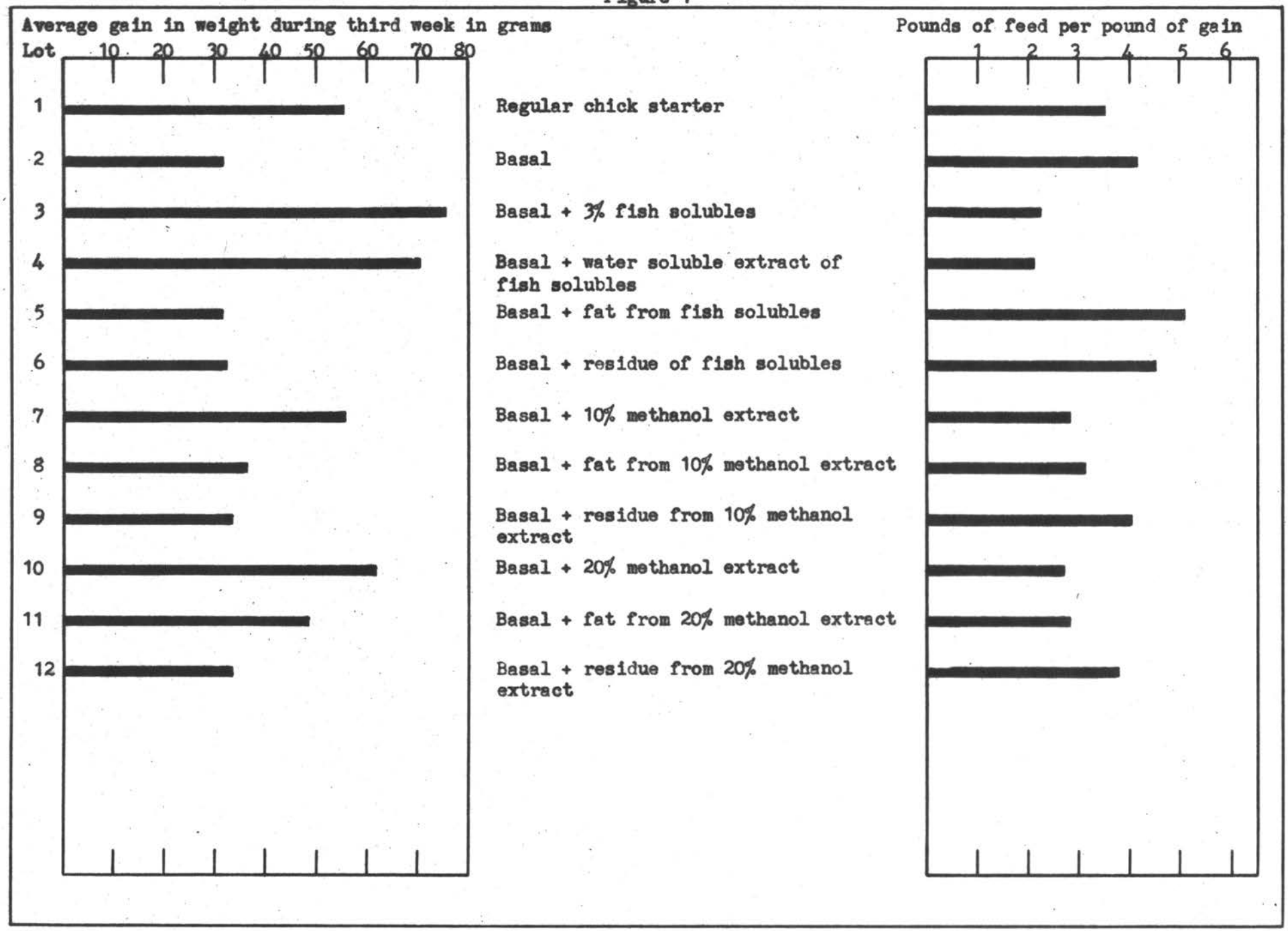
[4]Analyses made through the courtesy of Dr. Robert MacVicar, Professor, Agricultural Chemistry Research.

Table 1a

	Dry matter gms/100 ml.	Protein percent of dry matter	Ash percent of dry matter
Water soluble	2.53	67.5	29.0
Fat	3.58	25.3	3.8
Residue	3.56	40.9	5.7
Analyses of extraction made with fat and residue left together are:			
Water soluble	5.00	64.7	30.0
Fat and residue	10.84	31.1	1.6

A second group of chicks were hatched from the breeder hens after they had been fed an all-vegetable protein diet (Basal No. 2) for 2½ months. Hatchability on this group was 48.25 percent. Mortality during the first two weeks was 68.29 percent. It reached a maximum between the 5th and 10th days. A veterinarian examined the chicks, ran bacterial cultures, and made an autopsy. His report was general weakness and lack of nourishment. Food was present in the stomach and intestines of the chicks. Some secondary infection was observed. Chicks appeared weak and inactive at hatching time. Extreme pasting-up was observed in the chicks, starting about the sixth day. Eyes were pale and sunken. Most of the chicks showed normal eating habits.

Figure 1


Average gain in weight during third week in grams
Lot
10 20 30 40 50 60 70 80
1
2
3
4
5
6
7
8
9
10
11
12
Regular chick starter
Basal
Basal + 3% fish solubles
Basal + water soluble extract of fish solubles
Basal + fat from fish solubles
Basal + residue of fish solubles
Basal + 10% methanol extract
Basal + fat from 10% methanol extract
Basal + residue from 10% methanol extract
Basal + 20% methanol extract
Basal + fat from 20% methanol extract
Basal + residue from 20% methanol extract
Pounds of feed per pound of gain
1 2 3 4 5 6

EXPERIMENT 2

The chicks used in Experiment 2 were of the same origin as those used in Experiment 1. Since chick mortality had been very high in the previous trial, one percent of fish solubles was added to the basal diet (No. 1) for the first week of the pre-assay feeding period. The basal ration without the supplement was fed during the second week. Five-tenths percent of fish solubles had been added to the breeder mash (No. 2) in an attempt to increase hatchability. Mortality on 202 chicks during the first two weeks was 27.04 percent, as compared to 68.29 percent in the previous trial.

In experiment 2, attempts were made to precipitate the active factor from the water solution as obtained in Experiment 1. A 0.1 M solution of ammonium hydroxide and a 0.1 M solution of ferric chloride were used. Each precipitate was washed with the solution used to precipitate the factor, dried in a dryer, and fed in powder form as a component in the mash. The rates of growth obtained by feeding the ammonium hydroxide and ferric chloride precipitates are shown in Lots 3 and 4, Table 2, and Figure 2. These precipitates gave an increase in growth during the third week of 34.4 and 25.7 grams, respectively; whereas, the basal gave an increase of 37.3 grams. This would indicate that the growth factor was not precipitated out and was left in the combined ammonium hydroxide and ferric chloride filtrates which gave an increase in growth of 59.0 grams (Lot 5).

In Lot 6, the water soluble fraction was first acidified with 0.1 M Sulfuric acid before precipitating with ferric chloride. Little, if any, stimulation of growth was obtained by this precipitate. The

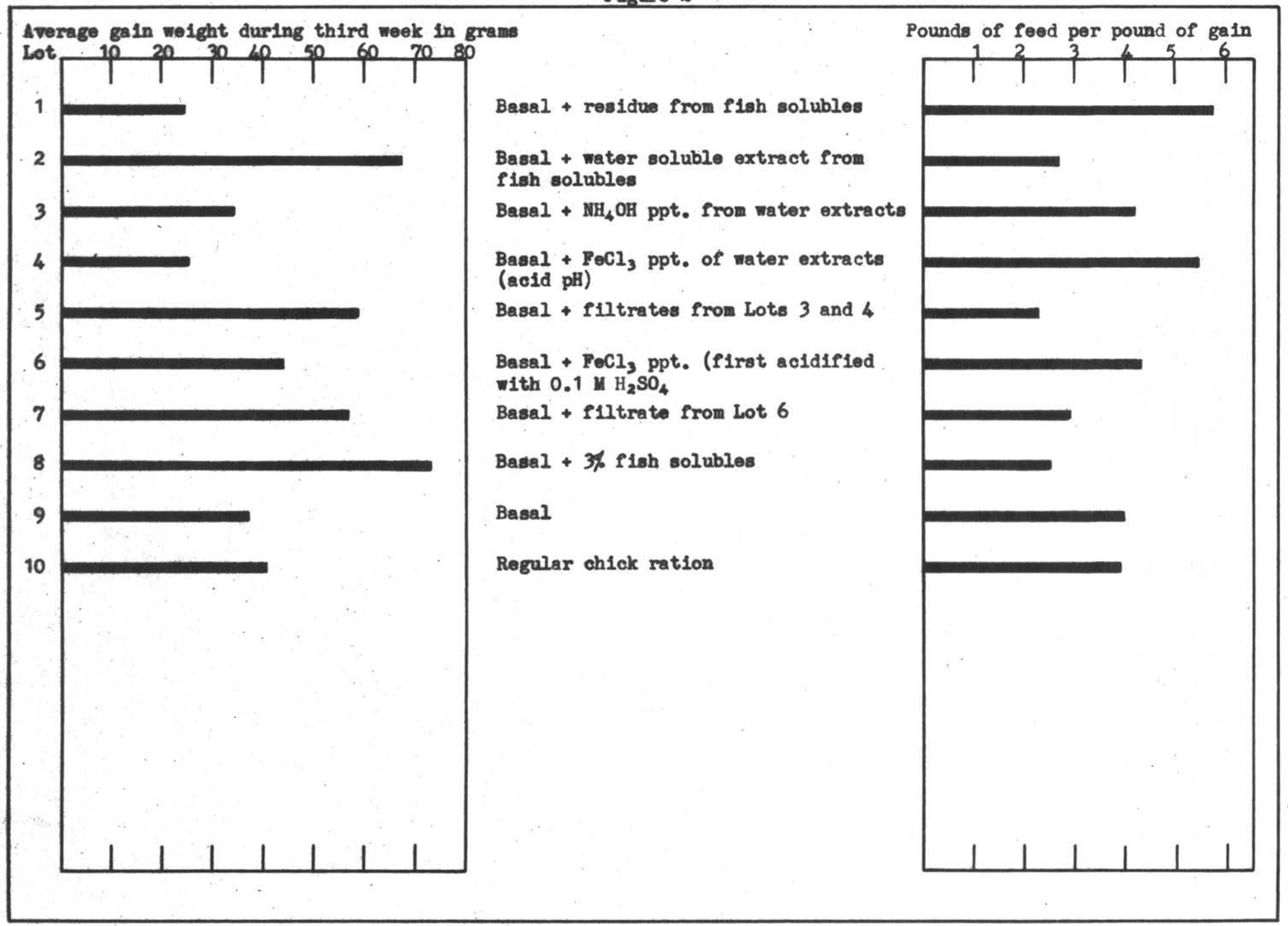
result obtained by feeding the filtrate (Lot 7) shows that the factor was not precipitated out by ferric chloride in an acid pH. The regular chick ration (Lot 10) which contained meat scraps, gave very little growth stimulation. Apparently this ration was low in some factor essential for growth.

As in Experiment 1, the water soluble fraction of the fish solubles (Lot 2, Table 2, and Figure 2) brought about an increase in the rate of growth. The residue, however (Lot 1) gave even less growth than did the basal itself (Lot 9). The efficiency of feed utilization was comparable in Lot 2 which contained the water soluble extract and in Lot 8 which contained the fish solubles, but greater than the basal itself (Lot 9). The pounds of feed per pound of gain in the three lots were 2.70, 2.55, and 4.02, respectively.

Table 2

Lots	Supplements	Ave. Gain 3rd Week	Lbs. Feed/ Lb. Gain
1	Basal + residue from fish solubles	28.4 gms	5.71
2	Basal + water extract from 3 percent fish solubles	67.6 gms	2.70
3	Basal + NH_4OH ppt. from water extract	34.4 gms	4.21
4	Basal + $FeCl_3$ ppt. of water extract (acid pH)	25.7 gms	5.44
5	Basal + Filtrates from Lots 3 and 4	59.0 gms	2.31
6	Basal + $FeCl_3$ ppt. (first acidified with 0.1 M H_2SO_4)	44.2 gms	4.33
7	Basal + Filtrate from No. 6	57.1 gms	2.94
8	Basal + 3 percent fish solubles	73.3 gms	2.55
9	Basal	37.3 gms	4.02
10	Regular chick ration	40.8 gms	3.93

Figure 2


Average gain weight during third week in grams
Lot
10 20 30 40 50 60 70 80
1
2
3
4
5
6
7
8
9
10
Basal + residue from fish solubles
Basal + water soluble extract from fish solubles
Basal + NH_4OH ppt. from water extracts
Basal + $FeCl_3$ ppt. of water extracts (acid pH)
Basal + filtrates from Lots 3 and 4
Basal + $FeCl_3$ ppt. (first acidified with 0.1 M H_2SO_4
Basal + filtrate from Lot 6
Basal + 3% fish solubles
Basal
Regular chick ration
Pounds of feed per pound of gain
1 2 3 4 5 6

EXPERIMENT 3

The chicks used for Experiment 3 were from breeder hens considered to be on an adequate, well balanced breeder ration. Half of the chicks were Single Comb Rhode Island Reds and half were New Hampshires. One percent of fish solubles was added to the basal ration (No. 1) for the first week. The basal ration without the supplement was fed during the second week. Mortality for the first two weeks was 10.9 percent, with 216 chicks on feed.

Precipitates of ferric chloride were again fed as in Experiment 2. However, the filtrates from each were fed separately instead of being combined. The precipitates of ammonium hydroxide and ferric chloride failed to stimulate the rate of gain to any appreciable extent over that obtained by the basal (Lot 10). However, the ammonium hydroxide and ferric chloride filtrates (Lots 6 and 8) gave an increase in growth of 67.5 and 54.7 grams, respectively; whereas the growth increase from the basal was 37.3 grams.

The growth response in Lot 1 was obtained by the addition of 0.1 gram of anthronilic acid per pound of feed. Reports indicate that anthronilic acid was administered to Japanese women in order to increase lactation. Since some workers believe the factor which increases lactation is also associated with growth, this acid was fed in order to see if a stimulation of growth could be obtained. From the data presented, anthronilic acid apparently depresses growth at the level tested.

In lots 2 and 3, water soluble extracts of fish solubles were fed at levels of 6 and 0.6 percent, respectively, to determine what effect

different levels would have on the rate of growth. The supplementation of 6 percent in Lot 2 gave an increase in growth of 72.9 grams. In Lot 9, which was supplemented with 3 percent of fish solubles, an increase in growth of 78.0 grams was obtained. Lot 3, with a level of 0.6 percent fish solubles added to the basal, gave an increase in weight of 52.0 grams. This indicates that a supplement of 3 percent of fish solubles to this basal approaches the level for optimum growth.

The regular chick starter used in Lot 4, which contained dried buttermilk and meat scraps, gave an increase in growth of 73.0 grams when supplemented with 3 percent fish solubles. The regular unsupplemented chick ration (Lot 10, Table 2, and Fig. 2) had given an increase of only 40.8 grams. Apparently the extra growth response obtained in Experiment 3 was due to the supplementation of 3 percent fish solubles or a growth factor had been transmitted from the hen to the chicks.

Table 3

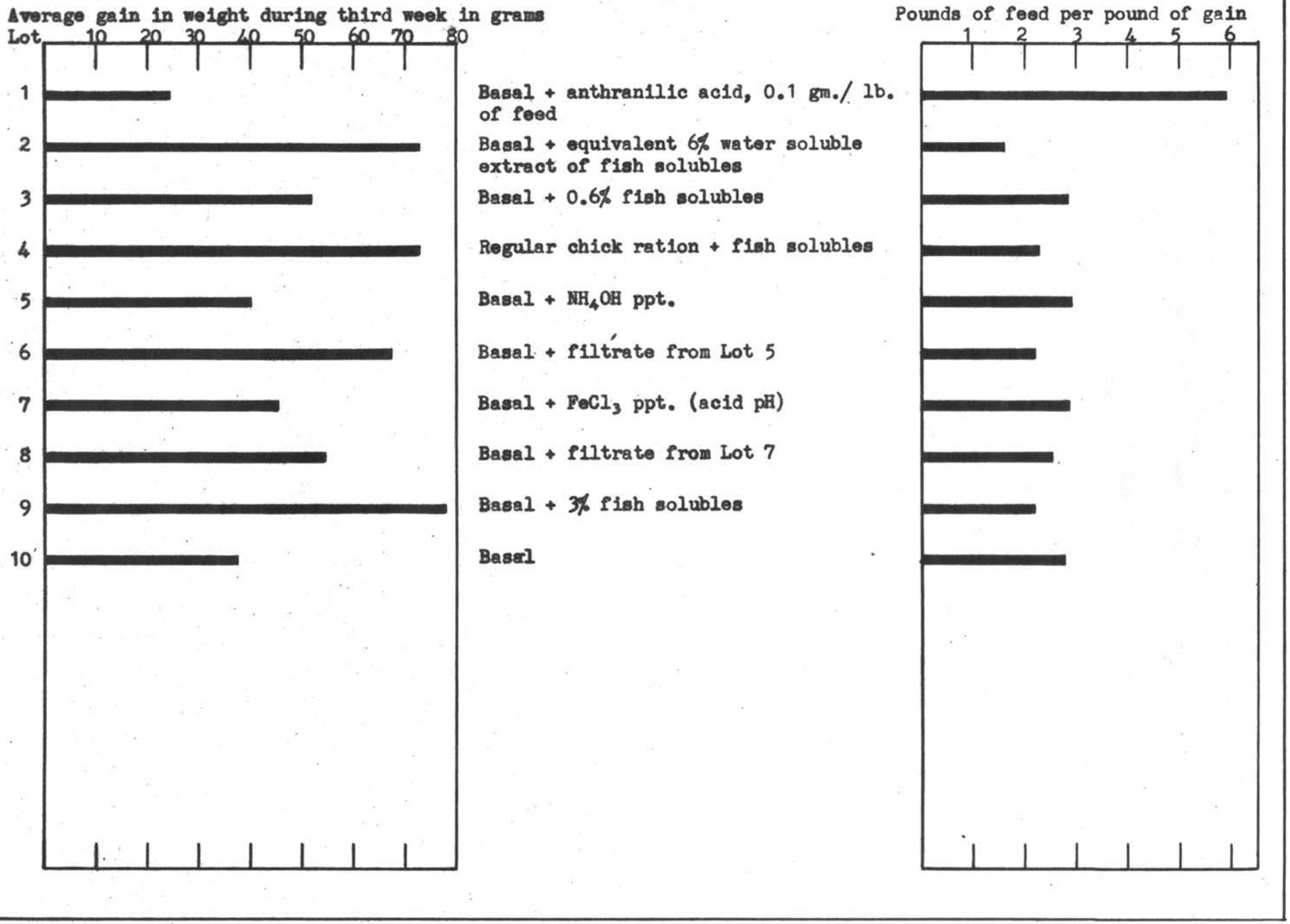
Lots	Supplements	Ave. Gain 3rd Week	Lbs. Feed/ Lb. Gain
1	Basal + Anthranilic acid, 0.1 gm/lb.	24.7 gms.	5.93
2	Basal + equivalent 6 percent water soluble extract	72.9	1.61
3	Basal + 0.6 percent fish solubles	52.0	2.87
4	Regular chick ration + fish solubles	73.0	2.30
5	Basal + NH_4OH ppt.	40.0	2.95
6	Basal + filtrate from No. 5	67.5	2.21
7	Basal + $FeCl_3$ ppt. (acid pH)	45.5	2.90
8	Basal + filtrate from No. 7	54.7	2.58
9	Basal + fish solubles	78.0	2.21
10	Basal	37.4	2.80

Analyses of the precipitates and the filtrates from Lots 5, 6, 7 and 8, are shown below.

Table 3a

Lots	Dry Matter gms./100 ml.	Protein percent of dry matter	Ash Content of Dry Matter
5		54.7	
6	2.85	86.03	3.31
7		55.75	
8	3.39	69.12	4.89

Figure 3


Average gain in weight during third week in grams
Lot
10
20
30
40
50
60
70
80
1
2
3
4
5
6
7
8
9
10
Basal + anthranilic acid, 0.1 gm./ lb. of feed
Basal + equivalent 6% water soluble extract of fish solubles
Basal + 0.6% fish solubles
Regular chick ration + fish solubles
Basal + NH_4OH ppt.
Basal + filtrate from Lot 5
Basal + $FeCl_3$ ppt. (acid pH)
Basal + filtrate from Lot 7
Basal + 3% fish solubles
Basal
Pounds of feed per pound of gain
1
2
3
4
5
6

EXPERIMENT 4

Chicks for this Experiment were from the same breeder hens as were used in Experiment 1 and 2. The breeder ration (No. 2) was supplemented with 0.5 percent of fish solubles. For the first week the chick's basal ration was supplemented with one percent fish solubles. Straight basal mash was used during the second week. Mortality on 132 chicks for the first two weeks was 11.4 percent.

The water extracts of fish solubles were concentrated under partial vacuum in order to simplify fractionations. This was done at a temperature of 55 to 60 degrees C. Attempts were made to dialyze the "growth factor" or "unidentified factor" through a cellophane membrane. A small amount of the water soluble extract was put into a short section of the cellophane bag. Then the bag was placed in a beaker of distilled water. The ends of the cellophane bag were tied firmly over the top of the beaker containing the distilled water. The water surrounding the cellophane bag was changed every 24 hours. This procedure was repeated eight times and the total amount of dialyzed material reduced under partial vacuum for feeding. Results (Lot 4, Table 4) indicate that the factor essential for growth was dialyzed through cellophane. Little, if any, growth resulted from the portion which did not dialyze through the cellophane (Lot 5, Fig. 5).

Since it is a recognized fact that some materials can be precipitated out by changing the electrical charge on its particles, an apparatus was set up in order to see what effect electrolysis would have on the unidentified factor(s). Two cellophane bags containing distilled water were placed in a beaker containing the water soluble

extract. A positive electrode and a negative electrode were made from nickel wire and coiled to increase the surface area. The two electrodes were placed in the cellophane bags containing the distilled water. The machine discharged 18 volts of direct current at each electrode. The contents of each cellophane bag was changed three different times after remaining in the set up from 10 to 12 hours. Apparently the unidentified factor(s) was not concentrated at either electrode (Lots 1 and 2) as shown by the growth obtained. Some of the factor might have been destroyed by this procedure. Some stimulation of growth did occur in the fraction which did not pass through the membrane by electrolysis (Lot 3); however, this is less than would be expected unless some of the factor had been destroyed. This assumption seems justifiable since the growth response in Lot 3 was less than that in Lot 4.

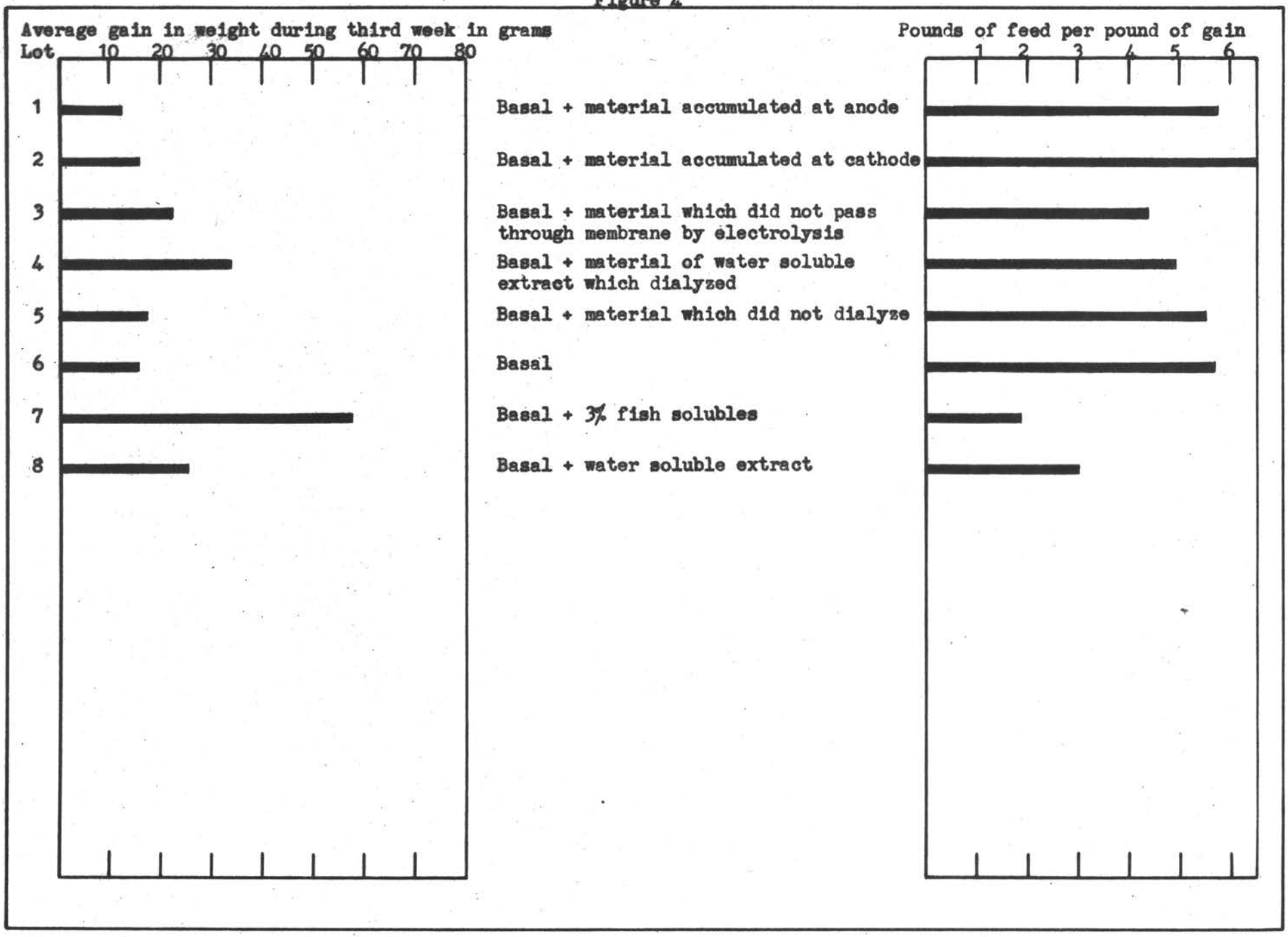
Fish solubles and the water soluble extract gave an increase in growth of 57.9 and 25.4 grams, respectively, (Lots 7 and 8). The difference in growth response obtained in Lots 7 and 8 cannot be accounted for unless it was because the water soluble fraction had been stored in a cooler for a period of three months. The basal (Lot 6) produced an average gain of only 15.8 grams.

The chicks in Experiment 5 consumed about half as much mash as did the same number of chicks on previous experiments during the third week. The feed consumption might have been influenced by the summer weather conditions.

Table 4

Lots	Supplements	Ave. Gain 3rd Week	Lbs. of Feed/ Lb. of Gain
1	Basal + material accumulated at positive electrode	12.2 gms.	5.77
2	Basal + material accumulated at negative electrode	16.0 gms.	6.57
3	Basal + material which did not pass through membrane by electrolysis	22.5 gms.	4.40
4	Basal + material of water soluble extract which dialyzed through membrane	34.0 gms.	4.93
5	Basal + material which did not dialyze	17.4 gms.	5.52
6	Basal	15.9 gms.	5.71
7	Basal + 3 percent fish solubles	57.9 gms.	1.89
8	Basal + water soluble extract	25.4 gms.	3.03

Figure 4


Average gain in weight during third week in grams
Lot
10
20
30
40
50
60
70
80
Pounds of feed per pound of gain
1
2
3
4
5
6
1 Basal + material accumulated at anode
2 Basal + material accumulated at cathode
3 Basal + material which did not pass through membrane by electrolysis
4 Basal + material of water soluble extract which dialyzed
5 Basal + material which did not dialyze
6 Basal
7 Basal + 3% fish solubles
8 Basal + water soluble extract

EXPERIMENT 5

Chicks used in this Experiment were from Rhode Island Red breeder hens considered to be on an adequate, well-balanced ration. For the first week the chick's basal ration was supplemented with one percent of fish solubles. Straight basal mash was used during the second week. Mortality during the first two weeks was 14.4 percent.

Various research workers have reported the precipitation of the unidentified growth factor(s) by the use of alcohol. In this Experiment two different concentrations of water soluble fractions were used in an attempt to precipitate the active factor. One fraction was not concentrated, while the other was concentrated under partial vacuum. Sixty percent ethanol was added to the water soluble fraction of each. The filtrate from the uncondensed portion (Lot 1) shows increase in growth of 45.8 grams during the third week. The 60 percent alcohol precipitate (Lot 2) gave an increase in growth of 49.5 grams. In the portion concentrated under vacuum, the filtrate (Lot 5) and the precipitate (Lot 6) gave an increase in growth of 51.6 and 51.8 grams, respectively. Since the filtrate and precipitate of each portion gave almost the same increase in growth, it appears that some of the factor was precipitated and some remained in solution.

In another procedure 70 percent ethanol was added to the water soluble fraction of both the uncondensed and condensed portions. The filtrate of the uncondensed portion, which was fed to Lot 3 gave an increase in growth of 51.6 grams; whereas the precipitate (Lot 4) gave a growth increase of 31.9 grams. The results in growth obtained by feeding the filtrate and precipitate from the condensed portion are

shown in Lots 7 and 8, respectively. An increase in weight of 71.8 grams occurred in the filtrate (Lot 7); however, the precipitate (Lot 8) gave an increase in growth of only 30.8 grams. This indicates in both the condensed and uncondensed portions that the factor was not precipitated by 70 percent ethanol. The precipitates (Lots 3 and 8) gave even less growth than did the basal itself (Lot 11, Fig. 5).

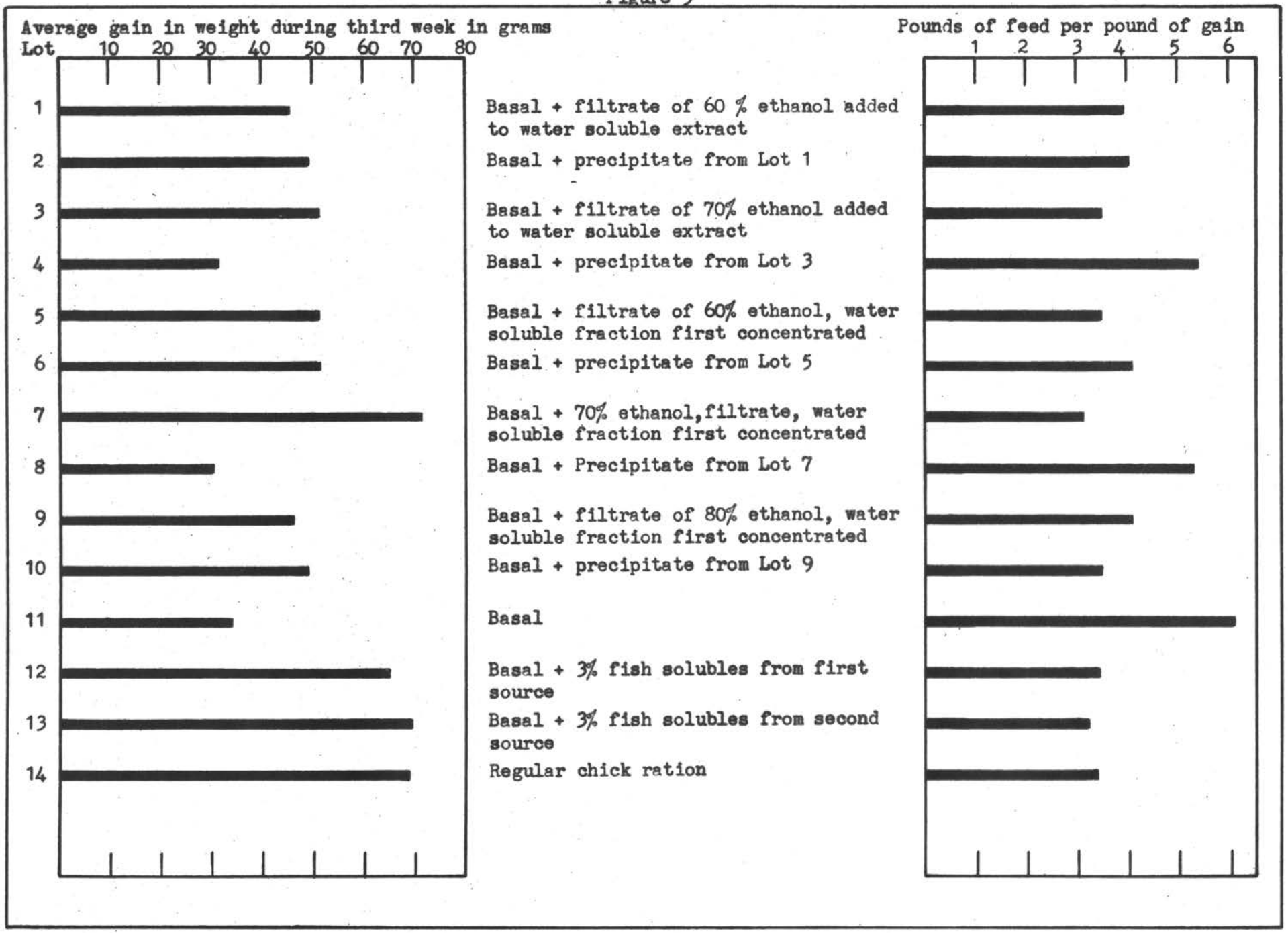
The rate of growth obtained by feeding the 80 percent alcohol filtrate and precipitate from the concentrated portion of the water soluble fraction are presented in Lots 9 and 10, respectively. Again the alcohol seemed to precipitate part of the growth factor. The filtrate (Lot 9) gave an increase in growth of 65.2 grams, and the precipitate (Lot 10) an increase of 49.1 grams.

Comparable growth rates were obtained by the fish solubles from different sources (Lots 12 and 13). The first source, Lot 12, gave an increase in growth of 65.2 grams and the second source, 69.7 grams. The small difference could be due to normal variation. The regular chick ration used in this Experiment, Lot 14, brought about an increase in growth rate of 69.1 grams.

Table 5

Lots	Supplements	Ave. Gain 3rd Week	Lbs. of Feed/ Lb. of Gain
1	Basal + filtrate of 60% ethanol added to water soluble fraction	45.8	3.97
2	Basal + precipitation from Lot 1	49.5	4.04
3	Basal + filtrate of 70 percent ethanol from water soluble extract	51.6	3.52
4	Basal + precipitation from Lot 3	31.9	5.41
5	Basal + filtrate of 60% ethanol added to (concentrated) water soluble extract	51.6	3.52
6	Basal + precipitation from Lot 5	51.8	4.12
7	Basal + filtrate of 70% ethanol added to (concentrated) water soluble extract	71.8	3.16
8	Basal + precipitation from Lot 7	30.8	5.31
9	Basal + filtrate of 80% ethanol added to (concentrated) water soluble extract	46.3	4.12
10	Basal + precipitation from Lot 9	49.1	3.51
11	Basal	34.2	6.11
12	Basal + 3% fish solubles (first source)	65.2	3.48
13	Basal + 3% fish solubles (second source)	69.7	3.26
14	Regular chick ration	69.1	3.42

Figure 5


Average gain in weight during third week in grams
Lot
10 20 30 40 50 60 70 80
Pounds of feed per pound of gain
1 2 3 4 5 6
1 Basal + filtrate of 60 % ethanol added to water soluble extract
2 Basal + precipitate from Lot 1
3 Basal + filtrate of 70% ethanol added to water soluble extract
4 Basal + precipitate from Lot 3
5 Basal + filtrate of 60% ethanol, water soluble fraction first concentrated
6 Basal + precipitate from Lot 5
7 Basal + 70% ethanol,filtrate, water soluble fraction first concentrated
8 Basal + Precipitate from Lot 7
9 Basal + filtrate of 80% ethanol, water soluble fraction first concentrated
10 Basal + precipitate from Lot 9
11 Basal
12 Basal + 3% fish solubles from first source
13 Basal + 3% fish solubles from second source
14 Regular chick ration

EXPERIMENT 6

Chicks for this experiment came from a flock of Barred Plymouth Rock Breeder hens which were fed a breeder mash considered to be adequate and well balanced. A pre-assay depletion period of only 12 days was used in this experiment instead of the usual 14 days as was done in the five previous experiments. Mortality on 358 chicks for the first 12 days was 13.7 percent.

A review of the literature indicates that growth is stimulated by the feeding of B-Complex vitamins as a supplement to an all-vegetable protein ration. Therefore, B-Complex vitamins were added to basal No. 1 in order to eliminate any possibility that a deficiency of these vitamins was depressing growth.

The following B-complex vitamin supplement was added to all rations including the basal fed during the pre-assay depletion period:

Table 5a

	Milligrams of Vitamins Added Per Lb. of Feed
Thiamin	0.9
Pantothenic acid	5.0
Niacin	8.0
Pyridoxine	1.6
Folic Acid	0.45
Inositol	100.0
Para-aminobenzoic acid	5.0
Choline Hydrochloride	700.0

A water soluble fraction of the fish solubles was prepared, dialyzed, and concentrated as outlined in Experiment 4.

The results of Experiment 6 are summarized in Table 6 and Figure 6. Vitamin B_{12}[5] was fed as a supplement in Lots 2, 5, 6, and 8 at a level of 7.5 grams per 1000 grams of feed. This level as reported by Merck's Laboratories[6] is the equivalent of 3 percent of fish solubles. Since workers believe that Vitamin B_{12} was fed to determine if the growth obtained by feeding it would be comparable to the growth response obtained by feeding the unidentified growth factor found in fish solubles.

The average chick weight obtained by feeding the basal ration plus the B-complex vitamin supplement (Lot 1) was 31.7 grams. Chicks on this diet required 5.16 pounds of feed per pound of gain. In Lot 2, Vitamin B_{12} was added to the basal ration containing the B-complex vitamins. The gain in weight (Lot 2) was 56.4 grams with only 3.30 pounds of feed consumed per pound of gain. The growth response obtained by feeding the dialyzable material from the water soluble extract (Lot 3) was 52.5 grams. This shows that the factor is the dialyzable portion of the water soluble fraction gave a growth increase comparable to that obtained from the addition of Vitamin B_{12} to the basal ration. The economy of gain between the two lots (Lots 2 and 3) was nearly identical. The growth increase obtained by feeding the undialyzable portion of the water soluble fraction (Lot 4) was 43.7 grams, as compared to 52.5 grams with the dialyzable fraction (Lot 3). Apparently a small amount of the growth factor was left in the undialyzable portion since

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[5]Vitamin B_{12} supplied through the courtesy of Merck's and Company and Dr. O. B. Ross, Professor of Animal Husbandry, Oklahoma A. and M. College.

[6]Personal communication to Dr. O. B. Ross.

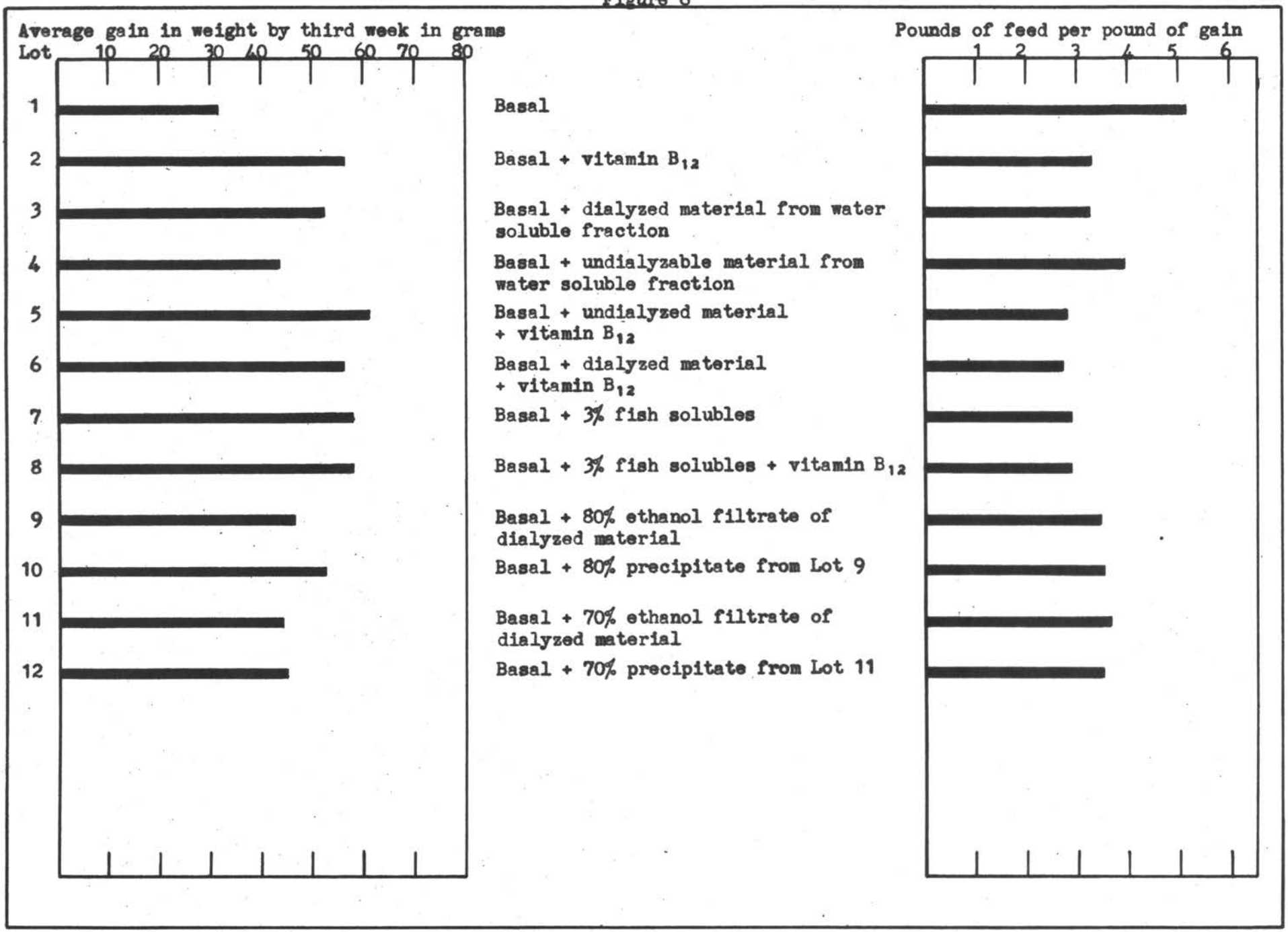
the increase in growth was 12.0 grams more than was obtained from the basal ration. However, the addition of Vitamin B_{12} to the undialyzable portion (Lot 5) gave a growth response of 61.3 grams. The amount of feed necessary for each pound of gain was the same as for the basal ration.

In Lot 6 Vitamin B_{12} was added to the dialyzed portion of the fish solubles. The growth increase was 56.3 grams, as compared to 56.4 grams for Lot 2 which contained the basal ration plus Vitamin B_{12}. The addition of 3 percent fish solubles to the basal ration (Lot 7) gave a growth increase of 58.0 grams. The growth obtained by adding both fish solubles and Vitamin B_{12} (Lot 8) was 58.2 grams. From these results, it can be concluded that a level of 3 percent of fish solubles supplies an adequate amount of the growth factor. This indicates also that Vitamin B_{12} is similar to the factor found in fish solubles.

Table 6

Lots	Supplements	Ave. Gain 3rd Week	Lbs. Feed/ Lb. Gain
1	Basal	31.7 gms.	5.16
2	Basal + Vitamin B_{12}	56.4	3.30
3	Basal + dialyzed material from water soluble fraction	52.5	3.27
4	Basal + undialyzable material from water soluble fraction	43.7	3.95
5	Basal + undialyzed material + Vitamin B_{12}	61.3	2.81
6	Basal + dialyzed material + Vitamin B_{12}	56.3	2.74
7	Basal + 3% fish solubles	58.0	2.90
8	Basal + 3% fish solubles + Vitamin B_{12}	58.2	2.89
9	Basal + 80% ethanol filtrate of dialyzed material	46.8	3.49
10	Basal + 80% precipitate from Lot 9	52.7	3.53
11	Basal + 70% ethanol filtrate of dialyzed material	44.3	3.68
12	Basal + 70% precipitate from Lot 11	45.1	3.52

Figure 6


Average gain in weight by third week in grams
Lot
10 20 30 40 50 60 70 80
Pounds of feed per pound of gain
1 2 3 4 5 6
1 Basal
2 Basal + vitamin B_{12}
3 Basal + dialyzed material from water soluble fraction
4 Basal + undialyzable material from water soluble fraction
5 Basal + undialyzed material + vitamin B_{12}
6 Basal + dialyzed material + vitamin B_{12}
7 Basal + 3% fish solubles
8 Basal + 3% fish solubles + vitamin B_{12}
9 Basal + 80% ethanol filtrate of dialyzed material
10 Basal + 80% precipitate from Lot 9
11 Basal + 70% ethanol filtrate of dialyzed material
12 Basal + 70% precipitate from Lot 11

DISCUSSION

The results obtained in this study consistently indicate that an unidentified growth factor is present in fish solubles which is essential for optimum growth, good livability, and high efficiency of feed utilization in chickens.

By all nutritional standards the basal ration fed contained all of the nutrients known to be essential for optimum growth. Nevertheless, the addition of 3 percent of fish solubles in all cases improved the growth rate.

From the data presented it is evident that the growth factor is not a known amino acid. The calculated analysis of the amino acid content of the basal ration shows that all of the essential amino acids were supplied in adequate amounts. Even the addition of methionine to a similar corn-soybean meal ration failed to give adequate growth (Emerson, 1948). Similar results were reported by McGinnis et al (1947).

Further evidence that the growth factor is not an amino acid is shown by the growth obtained from feeding the regular chick starter (Lot 10, Table 2). This ration contained "Liqua-Fish" at the equivalent level of 3 percent of fish solubles. Analyses of the amino acid content in "Liqua-Fish", as reported by the manufacturer, show that it is an excellent source of amino acids. The regular starter supplemented with "Liqua-Fish" contained amino acid levels well above the recommended requirements for optimum growth; yet, the regular chick starter failed to give satisfactory growth.

The growth factor was not identical to any of the B-complex vitamins which were added to the basal ration. The increase in the growth obtained was still below optimum, showing that some factor(s) essential for growth was still lacking (Lot 1, Table 6). Although an increase in growth was obtained when B-complex vitamins were added to the basal ration, even greater growth was obtained with the further addition of 3 percent of fish solubles.

The growth response obtained by feeding 0.1 grams of anthronilic acid per pound of feed shows definitely that it is not the growth factor. Apparently it produced detrimental effects since the increase in gain was less than was obtained from feeding the basal ration (Lot 1, Table 3).

The unidentified growth factor was contained in the water soluble extract of the fish solubles (Lot 4, Table 1). The active factor was also found to be soluble in 10 and 20 percent methanol as shown by the improved growth rates obtained by the addition of these supplements to the basal ration (Lots 7 and 10, Table 1). The growth response obtained from feeding the ammonium hydroxide precipitate indicates that the growth factor is not precipitated by this chemical. Chemical analysis indicates that both the ferric chloride and ammonium hydroxide precipitates are proteins, if it can be assumed that the nitrogen present is in the form of protein. In the precipitation procedure with ferric chloride some of the growth factor must have come down with the protein, since both the filtrate and precipitate gave better growth than was obtained with the unsupplemented basal ration (Lots 6 and 7, Table 2, and Lots 7 and 8, Table 3).

The active growth factor was not precipitated by 70 percent ethanol. However, 60 and 80 percent ethanol precipitated part of the factor. It is possible to conclude from these results that the concentration of the alcohol solution influences the solubility of the growth factor.

The factor was dialyzable through a cellophane membrane since the dialyzed portion stimulated growth equal to 3 percent of fish solubles (Lot 4, Table 4). Similar results were reported by McGinnis et al. (1947) and Zucker and Zucker (1948).

It is apparent that electrolysis had little, if any, effect in concentrating the growth factor from a water solution. Some of the active factor may have been inactivated, however. This assumption is valid when growth responses between Lots 3 and 5, Table 4 are compared.

A comparison of the growth rates obtained by feeding Vitamin B_{12} and fish solubles indicate that the unidentified growth factor and Vitamin B_{12} may be identical. The addition of 3 percent of fish solubles and the equivalent level of Vitamin B_{12} gave equal growth responses when added to the basal ration (Lot 2 and 7, Table 6). The feeding of both at the same time (Lot 8, Table 6) gave no increase in growth over that obtained by feeding either one separately in the basal ration (Lot 2 and 7, Table 6). However, the chemical structure of the growth factor found in the fish solubles will have to be determined before it can be proven conclusively that Vitamin B_{12} and this growth factor are identical.

Standard poultry rations considered to be adequate and well balanced often fail to promote optimum growth. The reason for this failure is difficult to explain. In practice the recommendation has been to add animal protein concentrates to poultry rations in order to supply essential amino acids and an unidentified growth factor(s) known to be essential for growth. In the past year or so, however, the value of this recommendation has been severely questioned. Processing methods employed by manufacturers of animal protein concentrates may destroy or preclude the growth factor. The regular chick starter used in these experiments contained meat and bone scraps and dried buttermilk in addition to all of the nutrients known to be essential for optimum growth and livability. However, from the growth obtained, in Lots 1, Table 1, and Lot 10, Table 2, it is apparent that some factor necessary for growth was lacking. The growth increase obtained from the regular chick starter in Lot 14, Table 5 indicates that the ration contained enough of the growth factor to support normal growth. Perhaps this ration (Lot 14, Table 5) contained meat and bone scraps from a different source than that used in the ration in the previous trial. It has been shown that a great variation occurs in the feeding value of different samples of meat scraps (Christiansen and co-workers, 1940).

In recent years packing plants have with-held the liver and glandular tissues from the meat and bone scraps. This causes a decrease in the feeding value of meat and bone scraps, since these glandular tissues are excellent sources of the growth factor.

"Liqua-Fish" contains both press cake and press water. Ordinarily press water is processed into condensed fish solubles. Since this is

true, "Liqua-Fish" should contain the growth factor in addition to the essential amino acids normally found in fish meal. However, the "Liqua-Fish" in the regular ration (Lot 10, Table 2) gave no better growth response than did the basal ration (Lot 9, Table 2). The addition of 3 percent of fish solubles to the basal ration in Lot 8, Table 2, however, gave nearly twice the growth response obtained with the unsupplemented basal ration.

Apparently the methods of processing are responsible for this difference in feeding value. The "Liqua-Fish" is heated and digested with sulfuric acid. The fish solubles are prepared in their natural form and the sulfuric acid added later as a preservative. It has been found that the growth factor present in cow manure is readily destroyed by autoclaving for one hour with 2 N acid, Bird et al (1948). Since the growth factor from cow manure and fish solubles are believed to be the same, the cooking of "Liqua-Fish" with sulfuric acid could destroy the factor. Perhaps part of the explanation as to why poultry rations are deficient in the growth factor could be explained on this basis. This assumption is borne out by the fact that the two products, fish solubles and "Liqua-Fish" are manufactured from essentially the same material and yet give such a big difference in growth response.

Hatchability and livability are determined in part by the ration fed to the breeder hens (Rubin and others, 1947). Since hens must transmit the growth factor through the egg to the chick, it is imperative that the breeder mash be fortified with adequate amounts of the growth factor in addition to the other nutrients known to be essential for hatchability. The breeder hens used to furnish the chicks in

Experiments 1, 2, and 4 were fed an all-vegetable protein diet. Hatchability was normal during the first 8 weeks immediately following the date the hens were placed on the all-vegetable protein ration. Mortality of the chicks hatched after the hens had been fed this ration for 8 weeks was 27.5 percent during the two week pre-assay period. At the end of 10 weeks, hatchability of the fertile eggs from these same breeder hens had decreased to 48.25 percent. The mortality of the chicks in this hatch was 68.29 percent during the two week pre-assay period. In the next experiment one percent of fish solubles was added to the basal ration fed during the first week of the pre-assay period. Five-tenths percent of fish solubles was also added to the breeder mash. Mortality during the two week pre-assay period decreased to 27.04 percent. After the breeder hens had been fed the supplemented ration for a period of one month the mortality of chicks from these breeder hens had decreased to 11.4 percent during the two week pre-assay period.

These results partially explain why rations considered to be adequate often fail to give good growth and good livability under practical feeding conditions. The breeder mash may contain insufficient amounts of the growth factor. Thus, the hens are unable to transmit the needed growth factor through the egg to the chicks. Consequently, the chicks from these hens have insufficient storage of the growth factor in their bodies. If they are fed a starter mash which is also deficient or marginal in the growth factor, poor growth and high mortality will result.

It was found from feeding tests in these experiments that a level of 3 percent of fish solubles added to the basal ration apparently was

adequate for optimum growth. The addition of 6 percent of the water soluble extract, gave no greater growth response when added to the basal ration (Lot 2, Table 3) than did the addition of 3 percent of fish solubles (Lot 3, Table 3). The addition of 0.6 percent of fish solubles gave less growth response than did 3 percent of fish solubles or 6 percent of the water soluble fraction. It is apparent that the addition of fish solubles at a 3 percent level approaches the optimum for normal growth, good livability, and high efficiency of feed utilization.

SUMMARY

1. An unidentified growth factor is present in fish solubles which is essential for optimum growth and livability in chicks and hatchability in hens.
2. The unidentified growth factor is not one of the following B-complex vitamins: Thiamin, Riboflavin, Choline, Niacin, Pyridoxine, Inositol, Para-aminobenzoic Acid, Pantothenic Acid, or Folic Acid.
3. The results obtained in this experiment indicate that the growth factor and Vitamin B_{12} may be identical.
4. The poor growth obtained with the basal ration was not due to an amino acid deficiency.
5. The unidentified factor is:
   (a) soluble in water, 10 percent methanol, and 20 percent methanol. It can be extracted from fish solubles with any one of these solvents.
   (b) dialyzable through a cellophane membrane.
   (c) not separated from a water solution by electrolysis.
   (d) not precipitated from a water solution by ammonium hydroxide and ferric chloride.
   (e) not precipitated from a water solution by the addition of 60, 70, and 80 percent ethanol.
   (f) partially adsorbed on the water-soluble protein precipitated by ethanol from a water soluble extract of fish solubles.
6. A 3 percent level of fish solubles in a poultry ration is near optimum for normal hatchability, growth and livability.
7. Manufacturing processes may destroy or preclude the unidentified factor from many animal protein concentrates normally considered

to be excellent sources.

8. A deficiency of this unidentified factor may be one explanation for the poor hatchability, poor growth, and poor livability often encountered under practical feeding conditions.

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