# SOURCES AND USES OF UNIDENTIFIED FACTORS FOR GROWTH AND PRODUCTION IN POULTRY

by

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#### INTRODUCTION

The unidentified factors are factors contained in a variety of products which when added to the diets of poultry improve the rate of growth, production, and/or hatchability.

Suggestions were made as early as 1929 that factors in animal products might be necessary for good growth of the chicken. Unknown factors present in animal products that were necessary for chick growth in addition to vitamin  $\rm B_{12}$  were recognized by many workers and were termed as "animal protein factors."

The unidentified factors required for optimum growth by both chicks and poults have been reported to exist in a wide variety of products, since the isolation of vitamin B<sub>12</sub> in 1948. These factors have been reported to stimulate growth rate of poultry when added to diets which were considered adequate with regard to essential nutrients.

The incorporation of five-year plans in India has given an impetus to poultry industry both in private and public sectors. During the last 20 years, the poultry industry in India has expanded to a considerable extent in response to the increasing demand of poultry meat and eggs.

Nutrition is very important in the production of poultry as health, growth and performance of the bird depends to a very large extent on the diets given. Feed also accounts for two-thirds of the cost of raising poultry.

Little or practically no research work has been done in the field of unidentified growth factors in India.

The purpose of this report is to study the sources, uses and importance of unidentified growth and hatchability factors in poultry nutrition and ascertain the possibilities of promoting the poultry industry in India.

## REVIEW OF LITERATURE

#### Growth

## Organic Factors.

"Whey factor." Johnson et al. (1942) found that a factor was present in casein and liver meal that was essential for growth of chicks. The factor was distinct from vitamin A, thiamine, riboflavin, nicotinic acid, pantothenic acid, paraeminobenzoic acid, choline and pyridoxine. The factor was found to be soluble in ethanol and was thermostable.

A series of experiments conducted by Csonka and Olsen (1949) showed that when chicks were fed high protein rations containing casein, they gained more in weight as compared to when fed low protein rations. The results of the experiment indicated that an unidentified growth factor(s) (hereafter referred to as UGF) was present in the diets fed. The carrier of this factor in the hen's diet would seem to have been casein as the most effective, corn as intermediate and soybean meal as the least effective under the experimental conditions.

Science News Letter (1951) quoted Picconi et al., from the University of Bologna, as having reported a new vitamin, or food element, to be present in crude casein and that it was different from vitamin B<sub>12</sub>. The factor was indispensable for normal growth and reproduction of rats. However, in a series of experiments conducted to study the effects of animal products on the growth of chicks and turkey poults it was shown the addition of vitamin-free casein or the amino acids--methionine, lysine and trypto-phane--to the basal diets, failed to give a growth response (McGinnis

et al., 1952).

Patrick (1953) working on unidentified growth promoting and fermentation factors in chicks stated that casein was a good source. The factor was said to be water soluble, unstable to acid treatment and prolonged enzymatic treatment. It improved the feather pigmentation and growth rate.

Davis et al. (1960) reported that 5% laboratory-produced dried whey significantly increased growth. Similar findings were obtained with equivalent quantities of deproteinized, delactosed dried whey and whey which in addition was demineralized with cation and anion exchange resins. Growth was also promoted by an equivalent amount (3.5%) of lactose. Crude lactose, recrystallized lactose, Baker reagent lactose and Pfanstiehl C. P. lactose, each promoted a growth increase of approximately 25% and was equal to that obtained with 5% dried whey. Little or no growth improvement was obtained with an equivalent amount of lactalbumen. The growth factor appeared therefore to be either adsorbed to a variable extent by lactose during crystallization or occluded on the crystals during growth.

Menge et al. (1949) found there was at least one unidentified factor which was not identical to vitamin  $\mathrm{B}_{12}$  and was required for rapid growth in the chick. The factor was said to be present in liver and dried whey. New Hampshire and Rhode Island Red chicks from dams receiving a diet containing no animal protein were used. When dried whey was fed in combination with vitamin  $\mathrm{B}_{12}$  crude concentrate, better growth was obtained than when vitamin  $\mathrm{B}_{12}$  crude concentrate was fed alone.

Dried whey, distillers dried solubles (hereafter referred to as DDS) and dried brewers yeast had been assayed by Couch et al. (1952) and were found to contain no appreciable quantity of  $B_{12}$ . It might be said the

growth promoting activity of whey, DDS and dried brewers yeast was not due to the vitamin B<sub>12</sub> content of the products. In experiments with New Hampshire broilers, it was noted that DDS, dried whey and whey products possessed another such factor which stimulated growth of chicks fed an all-vegetable protein ration.

Lillie et al. (1952) reported that dried whey had little effect on growth promotion, when compared to fish meal, fish solubles, meat meal and brewers yeast.

In four experiments conducted by Reed et al. (1951) different groups of birds were fed an all-vegetable protein diet (corn and soybean oil meal) and 3.5% dried whey was added either alone or in combination with animal protein factor (hereafter referred to as AFF) concentrate. Feeding of this product produced an increase in the weights of birds in all instances. This indicated that dried whey contains unidentified factors which are necessary for maximum chick growth.

The results of experiments conducted with chicks using different purified diets, Menge et al. (1952a) showed clearly that at least two distinct unidentified factors were required for rapid growth of the chick.

One of these was present in liver fraction L, liver fraction biopar C and dried brewers yeast, while the other was present in dried whey and not supplied in appreciable quantities by liver or dried brewers yeast. McGinnis et al. (1952) reported that whey and fish meal did not contain the factor present in liver fraction L or when present, the amount was insufficient to promote maximum growth.

Fisher et al. (1954) while working on alfalfa factor in relation to the separate unidentified factors present in defatted whole liver substance and in dried whey, both of which are known to enhance chick growth, suggested the above supplements contributed separate and distinct factors that were additive in their action.

Combs et al. (1954) reported in their work with day-old New Hampshire chicks that two UGF were required for rapid growth of chicks fed practical type rations. One of these was said to be supplied by butyl molasses fermentation solubles, butyl grain fermentation solubles, molasses dried distillers solubles, dried whey product and dried brewers yeast and the other by fish meal, crab meal, whale solubles and meat scrap. Orally edministered antibiotics had a sparing action on the dietary requirements for UGF. The use of antibiotics lessened rather than eliminated the need for dietary sources of these unidentified factors. Chicks fed practical type rations containing no added antibiotic did not respond in all trials to UGF supplements.

Combs et al. (1954) also stated that in about one-fourth of the trials conducted in batteries in three years no improvement in growth of chicks had resulted from various unidentified factor supplements. Hence certain conditions must have influenced the need for these factors. It seemed the critical need for the addition of the factors to a practical type ration was influenced by (1) Differences in body stores of the chick at hatching time.

(2) Factors that may influence the bacterial flora which becomes established in the intestinal tract of the chick. (3) Variations of ration ingredients in UGF content. (4) Multiplicity of unidentified factors involved with different ones limiting growth in certain trials and (5) Genetic and sex differences in the requirement of chicks for these factors.

In an effort to ascertain the value of adding several possible sources

of unidentified factors to the diet of the chick, Camp et al. (1954) conducted floor pen experiments using an all-vegetable protein basal diet containing all the known vitamins and minerals at levels known to be adequate for the chick and an antibiotic. The 10-weeks data strongly indicated that dried whey, a distillers fermentation product, and hydrolyzed whey contained an UGF for chicks, as growth rate was increased at a highly significant level. Furthermore fish solubles showed an entirely different growth factor which also initiated a highly significant increase in growth rate when compared with the basal diet. When fish solubles were fed in the presence of either of the above sources of the whey factor a highly significant increase in growth rate was noted when the weights were compared with the weight of the chicks receiving a source of only one of the chick growth factors.

In two experiments conducted by Kurnick et al. (1955), dried whey was studied as to its effect on growth in the later portion of the growing period. The birds were placed on a corn-soybean oil meal diet at 1 day of age. After periods of 67 and 86 days, respectively, in the two experiments, chicks were fed a purified type (Sucrose Drackett) diet for the remainder of the test. The addition of 4% dried whey (50% lactose) to the diet produced an increase in weight in both of the experiments.

Couch et al. (1955) stated that addition of 3% DDS to an all-vegetable protein diet produced an increase in growth (11.5%), and an improvement in feed efficiency, indicating that DDS contained the whey factor. The DDS and fish solubles, in combination, produced excellent results with regard to growth and feed efficiency in a practical type broiler feed. The feeding of DDS and dried whey also produced an increase in growth over that obtained with DDS alone and a corresponding improvement in feed efficiency. The data

obtained from feeding of DDS and dried whey indicated that the particular sample of DDS might have contained the fish factor or an additional UGF.

Dried whey, DDS and fish solubles increased the growth of chicks when added to an all-vegetable protein diet. The combination of fish solubles and dried whey stimulated the growth of chicks to a significantly greater extent than these supplements added singly (Camp et al., 1956).

Evidence obtained by Rassmussen et al. (1957) in experiments conducted to study the differentiation of UGF using a new semipurified ration, suggested that different unidentified factors were essential for rapid growth of chicks. (1) A factor present in dried whey and alfalfa leaf meal. (2) A factor present in DDS. (3) A factor present in Wilsons liver residue and fish solubles. With the exception of fish solubles each of these unidentified factor carriers appeared to contain lesser amounts of other factors. Fish solubles appeared to contain only liver residue factor.

Camp end Stephenson (1952) stated that corn molesses contained an UGF for growing chicks. White Wyandotte chicks of mixed sex were distributed at random among pens of a battery brooder. Two groups were fed a basel diet composed of soybean oil meal and ground yellow corn supplemented with all known required minerals, vitamins and a vitamin  $\rm B_{12}$ -eureomycin concentrate. The remaining chicks were fed five different diets with replicate pens receiving each diet. These five diets were identical to the basel diet except that two of them contained corn sugars, one contained a milo-molesses and two had corn molesses (Hydrol). Each of the sugars and molesses replaced 10 lbs. of corn (dry matter basis) in the diet. In the two experiments conducted, feeding of corn molesses increased the rate of growth significantly (p < .05). It also increased feed efficiency.

Data obtained from three floor pen feeding trials involving some 4,200 broiler type chicks presented by Camp et al. (1957) indicated: (1) The presence of two different chick growth factors one in dried whey and the other in fish solubles. (2) Presence of UGF in liquid corn steep water and its similarity to the factors in dried whey.

Russo and Hieman (1959) reported that significant increase in growth rate was found for chicks receiving 5% corn fermentation condensed solubles. They replaced an equal amount of corn meal or corn meal and scybean meal. No effect on feed efficiency was seen. Day-old White Leghorn Cockerels were divided into groups of 25 chicks each and housed in electrically heated battery brooders. Three replicates were used for each ration.

Working with fermentation byproducts of commercial origin and several prepared by a government research unit, Lillie and Denton (1959) reported that 4-week assays indicated that two samples were ineffective in stimulating a growth response either in the absence or presence of fish solubles, three samples were effective in the absence of fish solubles, and two samples were effective either in the absence or presence of fish solubles.

Tsang et al. (1960) used day old White Plymouth Rock cockerels from a commercial source in experiments conducted to evaluate corn fermentation solubles in poultry nutrition. The specific product used was corn fermentation solubles no. 3. They stated that corn fermentation solubles supplied UGF required by chicks for a rapid growth and provided utilizable protein and energy. It was found to replace equivalent amounts of fish meal in corn and soybean oil meal type rations with respect to UGF, and exerted greatest effect in stimulating growth of chicks in diets containing over 1,000 calories of productive energy per lb. of feed,

"Fish factor." The essential growth factor present in sardine fish meal and condensed fish solubles was reported by Pensack et al. (1949) to be soluble in 75% acetone. The factor was also stated to be coloriess in solution. Comparing vitamin  $B_{12}$  with fish solubles and liver products, Sunde et al. (1950) found that chicks fed fish solubles were larger at the end of the test period than those in the groups receiving crystalline  $B_{12}$  or  $B_{12}$  concentrate. It was therefore suggested that chicks fed all-vegetable rations required a factor(s) in addition to vitamin  $B_{12}$  and other vitamins for maximum growth.

Menge et al. (1952b) stated that a growth factor was present in condensed fish solubles and was found to be soluble in water, 50% and 60% alcohol and insoluble in ether. Most of the growth promoting activity was removed from fish solubles by extraction of 70% methanol or 80% ethanol. Feeding of essential amino acids in equivalent amounts as supplied by 4% fish solubles did not increase the chick growth response over that obtained with the negative control group. L-Lyxoflavin as well as indolacetic acid also failed to give a growth response indicating the chick growth factor present in fish soluble was not identical with these substances.

A series of experiments were conducted by McGinnis (1952) in an effort to study the effects of animal products such as fish meal, fish solubles, liver meal and dried whey on the growth of chicks and turkey poults fed different types of rations. Results obtained showed that (1) A combination of terramycin with whey and liver L gave a greater response than any of the supplements alone. (2) Though there were wide differences in the response to fish solubles added to the different soybean oil meals, these differences were of no significance since the interaction between meals and solubles was

not statistically significant.

Lillie et al. (1952) reported that in a 4-week assay for the unknown factor with New Hampshire male chicks, from hens fed a complete breeder diet, fish meal, fish solubles, meat meal, brewers yeast and dried cells of <u>Torula utilis</u> and <u>Hansenula suaveolens</u> were effective in promoting growth. Dried whey product and dried cells of <u>Streptomyces olivaceus</u> had little effect.

Results of two experiments conducted by Couch et al. (1952) showed the DDS, dried whey and whey products possessed an UGF which stimulated the growth of chicks fed an all-vegetable protein ration, and that fish meal possessed another such factor that stimulated the growth of chicks fed an all-vegetable protein ration. The evidence also indicated that live yeast culture promoted the synthesis of the fish meal factor in the gastrointestinal tract of the chick. Laboratory assays of dried whey, DDS and dried brewers yeast did not show the presence of appreciable quantities of vitamin  $B_{12}$ , and thus the growth promoting activity of the products was not due to  $B_{12}$  content of these products.

Wiese et al. (1953) working with day-old New Hampshire chicks, found that fish meal or fish solubles contained a growth promoting factor. The factor was also said to be present in dried whey but not in liver preparations. The chicks were fed purified diets for a period of 4 weeks. Increase in growth of chicks by about 50 gm. was noted by the addition of 3.6% fish solubles or 6% herring fish meal to the purified diet. Vavich et al. (1953) reported that sardine meal and brewers meal contained factors which produced growth stimulation.

A purified dist containing washed isolated soybean proteins, methionine, glycine, cerelose, soybean oil, pencillin, and all required minerals and

vitamins was used in several experiments with day-old straight-run New Hampshire chicks by Petersen et al. (1953) to determine the growth response from the addition of sources of unidentified factors singly and in various combinations. The results showed that (1) Fish meal, fish solubles, various whey products and some liver preparations resulted in growth increase. The growth increase over the basal at 4 weeks was approximately 15%. (2) Combinations of two or more supplements did not result in further growth improvement, indicating that under the conditions of their experiments the various products contained the same unknown factor(s).

Norris et al. (1953) reported that two UGF or a group of factors were present in fish meal and stated that DDS and dried liver also appear to have a second factor or group of factors. Results of the experiments conducted by Edwards et al. (1953) have provided further evidence of the existence of one or more unknown factors in fish solubles required for chick growth. Lots containing White Leghorn day-old male chicks were used in two experiments. Fish solubles promoted a marked increase in gain during the 4-week experimental period, and also reduced the amount of feed required per gm. of gain.

Berg et al. (1953) stated that addition of fish meal to purified type diets in chicks caused increase in growth.

Combs et al. (1954) in experiments conducted with chicks, found that two UGF were required for rapid growth of chicks fed practical type rations. One of these appeared to be supplied by fish meal, crab meal, whale solubles, and meat scrap, and the other by butyl molasses fermentation solubles, butyl grain fermentation solubles, molasses dried distillers solubles, dried whey product and dried brewers yeast.

Camp et al. (1954) have reported the presence of a growth factor in fish

solubles that produced a highly significant increase in growth rate compared to the basal diet of an all-wegetable protein type containing all known vitamins and minerals at adequate levels and an antibiotic. A second factor was present in dried whey, distillers fermentation product and hydrolyzed whey. Presence of either of these factors alone or in combination in the diet increased feed efficiency.

Various fish products and certain microbiological products were fed to chicks by Morimoto et al. (1955) to determine their value in comparison with fish meal. When autolyzed cuttle fish, autolyzed sardines and fish solubles, were fed to chicks, a growth response was observed that indicated the presence of UGF. However, none of these materials were found to be as potent as the growth factors present in fish meal.

Petersen et al. (1955) experimenting with day-old straight-run New Hampshire chicks fed a purified diet and observed that fish meal (6%), fish solubles (6%) and dried whey product (5%) all permitted about 15% increase in growth rate over the basal diet containing 20% protein. The sample of biopar C used in the study apparently did not contain activity. Combinations of one or more of the above substances did not result in increased growth indicating either that only one unidentified factor was required under the experimental conditions which was supplied by all the supplements or that if more than one was required, all products supplied them.

Tamimie (1955) stated that fish meal owed its growth promoting ability to at least two unknown factors, one of which could be extracted by water and was acetone soluble, and the second was an inorganic factor present in the ash of fish meal. The fish factor appeared to be identical to the liver factor. Fish solubles, dried whey and DDS were reported by Camp et al. (1956) to increase the growth of chicks when added to an all-vegetable protein diet. The combination of fish solubles and dried whey stimulated the growth of chicks to a significantly greater extent than did these supplements added singly.

Arscott (1956) reported that in experiments conducted with day-old New Hampshire male x Delaware female cross bred male chicks, fish solubles supplements fed at 4 and 8% levels produced growth responses.

Rand et al. (1958) observed a significant growth response with fish meals made in the laboratory from whole fish, fish muscle, fish solubles, and fish viscers. Fish bones, fish scales and skins had no apparent growth promoting effect. Meals made from whole fish produced greater growth response than fish solubles alone or meals without solubles. The growth response in fish meals was partly due to improvement in amino acid balance and partly due to an actual unidentified factor.

Experiments were conducted to determine the unidentified factor activity of three market samples of fish meal, three market samples of meat meal and one sample of condensed fish solubles in a diet containing either 2.5% dried whey or 2.5% of dried buttermilk as a source of whey factor activity by Summers et al. (1959a). The results of their experiment indicated that fish meal and meat meal were devoid of UGF, while the fish solubles gave a fairly consistent growth response. Summers et al. (1959b) reported that (1) Supplementing practical diets with 2.5% fish solubles permitted somewhat superior weights as compared to 2.5% dried whey when added to practical diets for chicks. (2) The combination of 2.5% dried whey plus 2.5% of fish solubles produced results which were essentially the same as those obtained with 2.5%

fish solubles alone. They stated that dried whey did not contain unidentified factor activity which was not supplied by fish solubles.

Levin et al. (1960) stated that defatted starfish meal can compete nutritionally and economically with the fish meal as a source of protein, calcium, and unidentified factors in poultry rations.

Menge et al. (1949) have stated that at least one unidentified factor which is not identical to  $\mathbf{B}_{12}$  was required for rapid growth in the chick. This factor was said to be present in liver.

Sunde et al. (1950), in experiments conducted comparing vitamin  $B_{12}$  with liver products, found that chicks fed these products were larger at the end of the test period than those in groups fed crystalline  $B_{12}$  or  $B_{12}$  concentrate. Hence they suggested that chicks fed all-vegetable rations required a factor(s) in addition to vitamin  $B_{12}$  and other vitamins for maximum growth.

Studies with fractions, obtained from refined liver paste dialysate by Combs et al. (1950), indicated the existence of four substances which promoted rapid early growth in chicks. Two of them were different forms of vitamin  $\mathbf{B}_{12}$  while the other two did not appear to be identical with vitamin  $\mathbf{B}_{12}$  or any of the known vitamins.

Menge et al. (1952a) reported that two distinct unidentified factors were required by the chick for rapid growth. One of these was supplied by a 70% alcohol insoluble portion of the aqueous extract of liver subjected to enzymatic action (fraction L) or by a similar 70% alcohol insoluble fraction of a hot water extract of whole liver or by dried brewers yeast (fraction bioper C), the other was supplied by dried whey.

Norris et al. (1953) and Petersen et al. (1953) reported that supplementing purified diets with liver, liver solubles or various preparations of liver resulted in growth increases in chicks.

Two experiments were conducted with chicks fed ration depleted and nondepleted in animal protein and alfalfa meal to test the response to UGF present in alfalfa meal, defatted whole liver and dried whey by Fisher et al.
(1954). They suggested that each of the three supplements contributed
separate and distinct factors. These factors were additive in their action.
Savage et al. (1950) as quoted by Fisher et al. (1954) found the residue of
a water extract of whole liver substance supported better growth than the
water extract. So it is likely that another factor(s) is present in whole
liver in addition to those present in liver L, which is the alcohol insoluble
fraction of the water extract of whole liver substance.

Edwards et al. (1955) reported that chicks required for normal growth two factors present in liver. Both of the factors were found in a 70% ethanol extract of concentrated water soluble material of liver; one was organic and the other probably inorganic in nature.

Addition of extra emount of arginine and tryptophan to the purified diet fed to chicks improved growth slightly but was not significant. Inclusion of dried liver, fish solubles, or both together in the basal diet increased growth by 21, 20 and 35%, respectively. This indicated that improved growth obtained by adding these crude sources of unidentified factors to the basal diet was not due to the presence of known nutrients in these materials, but rather to the unidentified stimulating agents. Dried liver fed at 5% level alone produced maximum growth response. Further various fractions were prepared from dried liver, which included liver residue, liver concentrate, liver fraction 1 and liver fraction 2. Feeding liver concentrate, liver fraction 1 and liver fraction 2 increased the growth

of chicks. Liver residue was inactive. One and one-half percent of the liver fraction 1 in the diet promoted maximum growth response.

Rassmussen et al. (1957) reported that a factor present in Wilson's liver residue and fish solubles was one of the three different unidentified factors essential for the rapid growth of chicks.

"Alfalfa or forage juice factor." The press juice of forage contained a factor for chicks (Kohler and Graham, 1951). This growth factor was not identical with vitamin B<sub>12</sub>, whey factor, vitamin B<sub>13</sub>, antibiotic effect or with other factors which may have been supplied by fermentation of AFF products. It was destroyed by drying whole forage crops. The factor gave a growth response in commercial chicks fed practical type rations.

Vavich et al. (1953) stated that dehydrated alfalfa contained one or more unidentified factors that stimulated the growth of chicks. The effect was enhanced when chicks were placed on a vitamin-depleted diet; the gains in weight being 78% more than those on control diet as compared to 20% gains when alfalfa was given from the beginning. Brewers meal and sardine meal contained factors which produced similar growth stimulation.

Scott et al. (1953) studied the growth stimulating ability of samples of sun cured and dehydrated alfalfa meal in promoting chick growth. Addition of six samples of alfalfa (three dehydrated and three sun cured) at 5% level significantly improved chick growth. Sun cured meal was superior to dehydrated meal.

Hansen et al. (1953) stated that chick growth was stimulated when either dehydrated or sun cured alfalfa was added to a purified diet containing all known factors. Dehydrated meal at a level of 10 to 20% gave a response comparable to that elicited by 5% of a forage juice concentrate. The factor

appeared to be more concentrated in sun cured meal than in dehydrated meal since the former gave its maximum response at the 5% level. They suggested the high temperature required to process dehydrated meal was responsible for decrease in activity.

Results of the studies by Fisher et al. (1954) indicated the factor(s) present in alfalfa meal was/were distinct from the factor found in either defatted whole liver or dried whey. The factor(s) did not appear to be related to the ash, amino acids or glucouronic acid present in alfalfa. Supplementation of the basal diet with alfalfa at 5% level gave a maximum growth response. The response was greatest and most consistent as compared to liver powder and dried whey.

Dehydrated cereal grass, dehydrated alfalfa and herring meal were tested by March et al. (1955) as sources of UGF for chicks fed a purified basal diet. The diet was calculated to be complete in all the known vitamins required for normal growth of chicks. In some experiments, chicks used were obtained from dams fed a natural breeder ration supplemented with dehydrated green feed and some chicks used were obtained from dams which had never received green feed in any form. They concluded that (1) Separate growth factors for the chicks were present in dehydrated green feed, and in herring meal. (2) The response to either of these factors was dependent upon the presence of the other in the ration. (3) The UGF present in dehydrated green feed was carried over from the dam to the chick.

Rassmussen et al. (1957) suggested that three different unidentified factors were essential for rapid growth of chicks. (1) A factor present in alfalfa meal and dried whey. (2) A factor present in Wilson's liver residue and fish solubles and (3) A factor present in DDS. With the exception of

the fish solubles each of these unidentified factor carriers appeared to contain lesser amounts of other factors, while fish solubles appeared to contain only the liver residue factor.

Three experiments were conducted by Liuzzo et al. (1960) to test the growth stimulating effects of concentrates prepared from dehydrated alfalfa leaf meal on chicks. The concentrates were added to the practical ration at a level equivalent to 5% alfalfa meal and fed ad libitum for 8 weeks. Significant growth responses, over the control, were obtained with concentrate A, B, and C. The results showed that all the three concentrates prepared from alfalfa leaf meal (which were active for Naurospora sytophila) were required for maximum growth of chicks.

Unclassified factor(s). Hopper et al. (1956) reported that fluid egg yolk improved the growth promoting ability of a corn-soy type basal diet. The egg yolk was obtained from the eggs of non-depleted hens. When supplemented, a purified diet, and a more practical corn-soy diet, stimulated chick growth during the last two weeks of a 28-day feeding period. Combining yolk with each of the three supplements used--defattened liver powder, dried whey and condensed fish solubles--resulted in slightly improved growth compared to that produced with these supplements alone. The egg yolk appeared to be lacking in the whey factor and it was suggested that it owed its major growth promoting ability to the fish factor contained therein.

Arscott (1956), conducting four experiments, fed fish solubles, egg yolk and animal fat in a basal ration containing sucrose and casein. He reported that animal fat and fish solubles contained the same UGF. Egg yolk on the other hand appeared to be composed of at least one additional growth factor. Existence of at least three factors has also been proposed by several

investigators, Kohler and Graham (1951), Fisher et al. (1954) and Combs et al. (1954).

Studies have been conducted by Arscott et al. (1957a) involving egg yolk components, types and levels of yolk, and dried yolk extraction using a sucrose-soybean meal diet. They stated that linoleic, palmitic and stearic acids, sodium cleate, cholesterol or an ashed yolk fraction (550° C) fed to chicks at levels equivalent to 8% dried yolk appeared to contain no growth stimulating effect. Soybean lecithin (1% Alcolec S), orotic acid (40-80 mg./kg.) or adinosine (300 mg./kg.) were also without effect. Near optimum chick growth responses occurred when 8% dried yolk was included in the diet when compared with the 4% and 12% levels. Four, 8, or 12% fluid chicken or turkey yolk also responded similarly.

In the investigations conducted by Wiese et al. (1960) using chicks, it was found that addition of 7.5% dried egg yolk resulted in a growth increase of approximately 12%.

Heuser and Norris (1951) described that animal protein feeds contained an unknown nutritive factor which was dissimilar to vitamin  $\mathbf{B}_{12}$ . Comparisons were made in the growth of chicks fed vegetable protein diets to which adequate amounts of vitamin  $\mathbf{B}_{12}$  were added, with the growth of chicks fed animal protein diets. Growth in chicks fed the animal protein diet was more than in the other group. This showed that the increases in growth were not due to vitamin  $\mathbf{B}_{12}$ .

Calf thymus, as reported by Ross et al. (1955), produced a growth response in chicks. In a series of 17 battery experiments conducted with White Plymouth Rock chicks feeding a 5.3% fresh calf thymus resulted in significant increase in weight gains, the average being 4.2% in chicks 3 to 6 weeks of age. They

were given a corn-soybean oil meal ration adequately fortified with all known nutrients.

Poultry byproduct meal, feather meal, and hydrolyzed poultry manure were found to be as effective as fish meal in commercial type broiler rations fed to broilers under practical conditions (Fuller, 1956).

Young et al. (1953) stated that alcohol extracted peanut meal supplied an UGF needed for chicks which is deficient or lacking in a purified casein-starch diet. Repeated experiments with cross bred male chicks (Rhode Island Red and Barred Plymouth Rock) fed a basal diet composed largely of corn starch and purified casein (25%) plus methionine and glycine failed to show optimum growth when given graded levels of arginine up to 2.06%. Addition of various amounts of alcohol-extracted peanut meal replacing equivalent amounts of casein protein promoted a marked growth response over that obtained on the basal diet supplemented with levels of arginine equal to those supplied by the peanut meal.

Patrick (1953) reported that soybean meal and casein were good sources of an unrecognized factor that improved feather pigmentation, growth rate and feed efficiency. Peanut meal, sesame meal, meat scrap and corn gluten were found to be poor sources of this factor. It was water soluble, unstable to acid treatment, and was destroyed by prolonged enzymatic treatment.

Kratzer et al. (1959) reported that an organic growth promoting and antiperotic factor(s) was/were present in soybean oil meal which could be extracted with methanol, but was insoluble in acetone. They also stated the growth promoting activity of brain powders and egg yolks was concentrated in their solvent extracted residues.

Edwards et al. (1953) stated that Penicillin mycelium residue appeared

to be qualitatively comparable to fish solubles as a source of the chick growth factor(s). The percentage of increase due to the inclusion of fish solubles and <u>Penicillin</u> mycelium residue in the basal diet was approximately the same at all comparable levels in two experiments conducted. Inclusion of procaine penicillin in the basal diet promoted an 11.4% increase in weight over the lot fed the basal diet only. The responses obtained with <u>Penicillin</u> mycelium residues were due if any, in small part, to penicillin content.

The fermentation material produced by culturing Streptomyces species B-1354 on suitable media was found to possess marked growth stimulating activity by Fritz et al. (1955). The data indicated the growth stimulation was due to an unidentified factor rather than the vitamin  $\mathbf{B}_{12}$  and antibiotic activity which this organism also possesses.

Schaffer et al. (1955) reported that <u>Penicillin</u> and <u>Streptomycin</u> mycelium meals contained at least two unidentified chick growth factors. Feeding of a combination of <u>Penicillin</u> mycelium and <u>Streptomycin</u> mycelium stimulated growth equal to or greater than that obtained with fish meal, yeast, DDS, whey and liver. Levels of 3% <u>Penicillin</u> mycelium or 3% <u>Streptomycin</u> mycelium resulted in chick growth increments of 13% and 22%, respectively.

Rubin et al. (1946), conducting three feeding experiments with chicks observed there was practically an equal stimulation of growth by urine-free chicken feces and cow-manure. Only a slight stimulation of the growth was apparent in the group fed the urine containing hen manure. Apparently the urine constituents in the manure inhibited growth to some extent.

Halbrook et al. (1950) reported addition to the basel diet of 1%, 2.5% and 5% levels of screened unautoclaved built-up corn cob litter (over 1 year

old) gave increased chick growth to 6 weeks of age of 74, 75, and 67 gm., respectively, as compared to 84 gm. for the added APF supplement fed at the rate of 20 u/gm. of vitamin  $B_{\gamma \gamma}/kg$ . of diet.

Elam et al. (1954) fed New Hampshire chicks autoclaved poultry litter suspension at a level of 8 ml. per lb. of feed and also in combination with fish solubles and with a combination of antibiotics. Growth of chicks was increased by the addition of litter-suspension, the antibiotic combinations and fish solubles. Growth was further increased when the autoclaved litter suspension was fed in combination with fish solubles or the antibiotic combinations.

Wehunt et al. (1960), as a result of four chick feeding trials, found that autoclaved poultry manure was approximately equal to condensed fish solubles and DDS combined, and growth was superior to either alone, in supplementing corn-soybean oil meal type rations containing no other UGF supplements. The hen manure was obtained from caged layers fed a complete laying mash containing no drugs. It was fed to chicks at levels of 1.5 and 3% of the diet, and compared with 3% condensed fish solubles and 1.5% distillers solubles fed singly and in combination.

Carver and Johnson (1953a) conducted experiments with chicks maintained on industrial soybean protein basal that contained an average of 0.04% fat and concluded that chicks required factors present in unsaturated fats for maximum growth. Crude corn oil, refined corn oil, soybean oil, wheat germ oil, oleic acid, and oleic acid concentrate contained these factors in varying concentrations. Wheat germ oil and the oleic acid concentrate appeared to contain relatively large quantities of the factors as they were superior to other unsaturated oils in stimulating growth. The UCF in unsaturated fats

were distinct from the growth factors in a fat free commercial liver concentrate biopar C (Carver and Johnson, 1953b). These same workers also state that pork liver residue, with arachidonic acid, constituting 18.8% of its total fatty acid content, was a fair source of ether soluble growth factors.

Ross et al. (1961) reported that water soluble portion of swine intestinal mucosa significantly improved the growth of chicks, and was not identical with any of the nutritional factors known to be essential for chick growth.

## Inorganic Factor.

Experiments conducted by Scott et al. (1955) show that growth promoting power of a basal diet was improved by supplementation with ash of distillers solubles; whereas, the solubles themselves consistently improved chick growth.

The ash of DDS was found by Reid et al. (1955), Dannenburg, et al. (1955), and O'Dell et al. (1957) to increase chick growth.

Experiments were conducted by Morrison et al. (1955b), using corn DDS, fish solubles, grass juice, dried whey product and <u>Penicillin</u> mycelium and ash of mixture of the UGF supplements. The results of the experiments showed that (1) A portion of the growth response obtained when a mixture of unidentified factor supplements were fed to chicks was due to a mineral constituent(s) in the mixture, (2) Addition of all trace minerals known to be required by the chick or alteration of the calcium and phosphorous content of the diet did not influence growth, and (3) The active components of the ash of UGF supplements as a mineral not yet recognized as essential.

Lillie et al. (1955) obtained evidence that indicated there is present an UGF in feather meal which may be inorganic in nature. These workers conducted two experiments using day-old New Hampshire (broiler) chicks in lots of 50 each, and in 4 replicates of 200 each in the first and second experiments, respectively. In the first experiment, the addition of 5% feather meal produced a growth response and feed efficiency value equivalent to that on 5% fish meal at 10 weeks of age. This finding was confirmed in the second experiment in which 4% fish meal and 3% feather meal were tested.

Morrison et al. (1955a), in experiments with chicks, found that increased quantities in the diet adequate in recognized nutrients of known vitamins, essential amino acids or known essential minerals (K, Na, Ca, Mg, P, Cl<sub>2</sub>, Fe, Cu, I<sub>2</sub>, Mn, Mo, Zn) failed to increase chick growth. The neutralized ash of a mixture of sources of unidentified factors or the ash of a composite sample of DDS increased chick growth. The same authors (Morrison et al., 1956b) while studying the isolation of the chick growth promoting factor(s) in the ash of UGF supplements reported that ash of UGF supplements significantly increased growth at 4 weeks of age when added to purified diets containing adequate amounts of nutrients required.

Using day-old male progeny of a sex linked cross of New Hampshire males and Silver Cornish females, Menge et al. (1956) showed that feather meal and feather meal ash stimulated growth that was significant at the 1% level.

The chicks were fed a complete diet and feather meal ash. They stated the UGF present in feather meal was/were inorganic in nature.

Camp et al. (1956) reported that the inorganic constituents obtained from fish solubles or DDS by ashing in a muffle furnace at 600° C produced a highly significant increase in growth of chicks. The growth response was equal to about one-half of that obtained with the untreated product in case of fish solubles and the growth response obtained with DDS ash was equal to that obtained with the untreated product. They also found that addition of a mineral mixture (reconstituted ash) which simulated the composition of a 1:1 mixture of dried whey ash and fish solubles ash, to the basal diet produced a highly significant increase in the growth of chicks to 10 weeks of age on a practical diet. It has been reported by Supplee et al. (1956) that supplementation of a highly purified basal diet (washed Drackett C-1 assay protein-sucrose type) fed to the chicks with the ash of certain unknown factor supplements resulted in improved gains averaging about 5%. The ash of a mixture of condensed fish solubles, DDS, distillers dried yeast, dried whole whey and molasses distillers dried solubles improved growth in poults fed a similar purified diet.

Morrison et al. (1956a) stated that when a mixture of UGF supplements were fed to chicks the growth response observed was due to the presence in the materials of both unidentified organic and inorganic constituents, and that a mineral(s) not considered to be essential in the nutrition of the chick was/were present in the ash of certain crude feed stuffs. They also stated that unknown mineral nutrient(s) was/were present in the boiling water-insoluble fraction of the ash of a mixture of unidentified factor supplements.

March et al. (1958) working with New Hampshire cockerels of one strain, but obtained from dams fed different diets and kept under different conditions of management, found the ash of whole eggs on chicks, obtained from dams fed a depletion diet and kept on wire resulted in a significant stimulation to growth when added to the basal diet.

Mason et al. (1961) reported that both inorganic and organic compounds were involved in the growth response that was observed when chicks were fed diets containing condensed fish solubles and the necessary inorganic constituents included copper and molybdenum.

#### Production and Hatchability

Egg production was improved by feeding dried whey and liver fraction L, by the injection of liver extract and by the feeding of a high level (5%) of an APF concentrate. The above mentioned products were assumed to contain an unidentified factor which was required for egg production (Couch et al., 1950). They stated that liver L contained a factor necessary for hatchability in addition to vitamin B<sub>12</sub> which was required for hatchability after the twelfth week.

The addition of 4% dried whey (50% lactose) to the corn-soybean oil meal diet of Single Comb White Leghorn pullets produced an increase in egg production (Kurnick et al., 1955). The hatchability of eggs was not affected by the dried whey supplements.

Experimental findings of Norris (1955) have shown a satisfactory hatchability by the addition of dried whey, dried whey products, fermentation products, dried yeast, DDS, fish meal, fish solubles, meat scrap, meat and bone scrap, liver meal and alfalfa leaf meal in various combinations to the breeder rations.

Lindstrom et al. (1949) in their experiments with 96, 6-month old White Leghorn hens, found there was an increase in hatchability within a period of 12 to 18 days by the addition of fish meal. This suggested the presence of unidentified factors in fish meal. The hens were fed soybean oil meal diet for three months. They were then divided into 6 lots of 8 hens each and fed 5 levels of fish meal varying from .00 to 4.5%. During the 42-day period of

fish meal supplementation the eggs produced during each 6-day interval were incubated and the percentage hatchability determined. Data indicated that 0.66 to 1.5% fish meal was sufficient to supply an adequate amount of the unidentified factor to permit an increase of 10-75% hatchability.

Factor L found in water soluble liver concentrates and fish solubles was reported to exert a strong influence on the number of hatchable eggs (Science News Letter, 1953). Diets without L factor reduced hatchability to 25% or less of the normal, and addition of the factor to the diets increased hatchability by 10% or more of the normal.

Stephenson and Clower (1952), in an effort to study the effect of fish meals on hatchability, fed one group of hens an all-vegetable diet, and the other a similar diet plus 3% condensed fish solubles. Within four weeks after the beginning of this study, the hatchability of fertile eggs produced by the hens receiving the all-vegetable diet had dropped to 15%. No change was observed in the hatchability of eggs from the hens receiving condensed fish solubles. Progeny studies also indicated a decreased hatching weight and retarded growth rate in the chicks produced by hens fed the all-vegetable diet. Arscott and Combs (1953) reported the presence of an unidentified hatchability factor in condensed fish solubles.

Alfalfa leaf meal, when fed in combination with vitamin  $\mathrm{B}_{12}$ ; increased hatchability over and above that obtained with vitamin  $\mathrm{B}_{12}$  alone (Jacobs et al., 1953). Addition of 5% dehydrated alfalfa meal to an all-vegetable protein diet containing vitamin  $\mathrm{B}_{12}$  increased hatchability when alfalfa meal was added either with or without vitamin  $\mathrm{B}_{12}$ . This indicated that dehydrated alfalfa leaf meal contained an unidentified factor necessary for hatchability and fertility. The number of embryos exhibiting vitamin  $\mathrm{B}_{12}$  deficiency

symptoms was not decreased when alfalfa leaf meal was added to the diet thus suggesting that alfalfa leaf meal does not contain vitamin  $B_{1,2}$  activity.

The addition of forage juice concentrate resulted in an increase of approximately 8% in number of saleable chicks (Stephenson et al., 1954). White Wyandotte hens were fed corn, soybean oil meal type all mash basal diets containing all known identified factors required by the breeding hens. Four lots of 35 hens each were fed only basal diet and four similar lots the basal diet plus 3% forage juice concentrate. The major difference in hatchability appeared to be due to reduction in the number of infertile eggs as determined by candling.

In studies conducted by Shutze et al. (1959) in an effort to determine unidentified nutritional factors affecting size, addition of 5% of wheat germ oil, safflower oil, or corn oil to a semipurified diet significantly improved egg size, but fish oil significantly decreased the egg size.

Whiteside et al. (1961) supplemented an all-vegetable protein diet containing chlortetracycline and penicillin with a fermentation residue, DDS, tetra alkyl ammonium stearate, condensed fish solubles, dried whey, molybdenum and zinc to study the effects on egg production, egg weight, feed efficiency and body weight of laying hens. They concluded that

(1) Significant increases in egg production were obtained when the fermentation residue, DDS, condensed fish solubles, dried whey or a combination of molybdenum and zinc were added to the basal diet; (2) Supplementation of the basal diet with combination of zinc and molybdenum or with single supplements of fermentation residue, DDS, condensed fish solubles and dried whey resulted in improved feed utilization, calculated on the basis of 1b. of feed per dozen 24-oz. eggs; (3) Groups receiving diets containing fermentation

residue, DDS, or dried whey as supplements to the basal diets gained from 27 to 69 gm. more in average body weight per hen than did the basal group.

Groups receiving tetra alkyl ammonium stearate, condensed fish solubles, or a combination of zinc and molybdenum as supplements to the diets gained from 19 to 54 gm. less per hen than the basal group.

#### SUMMARY AND CONCLUSIONS

A much investigated problem in poultry nutrition is the importance of unidentified factors present in certain substances. These factors are present in some feed stuffs and other substances which when included in the rations of poultry cause increased growth, or increased egg production and hatchability rate. In some cases they enhance the feed efficiency.

A survey of the literature has shown the unidentified factors can be classified as per the similarity of these factors as follows:

- 1. Unidentified factors required for growth.
  - A. Organic Factors.
    - (1) "Whey factor,"
    - (2) "Fish factor."
    - (3) "Alfalfa or forage juice factor."
    - (4) Unclassified factors.
  - B. Inorganic Factors.
- 2. Unidentified factors required for egg production and hatchability.

There is increasing evidence to show that most of the feed ingredients contain one or more factors that are like the one present in other ingredients. Alfalfa meal and dried whey are classified under separate groups but they possess the "whey factor" in common. Dried brewers yeast contains both

the "whey factor" and the "fish factor." Liver is reported to possess a "fish factor" and a factor similar to that present in dried whey and alfalfa meal.

At least two unidentified factors are necessary for optimum chick growth. Combination of two or more substances containing the unidentified factors are more effective in stimulating growth than with a higher level of each.

The type of the basal diet used also influences the activity of the unidentified factors. Better growth is obtained when the protein in the diet is of animal origin than when it is of vegetable origin. According to some reports the energy level of the ration influences chick response to the unidentified factors.

Purified diets are of much use in the study of unidentified factors.

Much further work has to be done before the exact nature of these
unknown factors can be established.

Substances containing the unidentified factors can be used to promote growth and increase production and hatchability and thus play an important part in poultry nutrition and poultry industry at large.

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# SOURCES AND USES OF UNIDENTIFIED FACTORS FOR GROWTH AND PRODUCTION IN POULTRY

by

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KANSAS STATE UNIVERSITY Manhattan, Kansas The purpose of this report was to study the importance of substances possessing unidentified factor activity in poultry nutrition, and evaluate the possibilities for improving growth and productivity of poultry in India where the tropical climate with high variation in temperature and rainfall causes a degree of stress in poultry limiting their production.

A review of the more recent literature of the unidentified factors for growth and production in poultry, mostly since 1948 when vitamin  $\rm B_{12}$  was isolated, revealed the following facts.

Certain unknown factors exist in some feed stuffs and a wide variety of other products. These factors stimulate the growth rate in chicken when added to diets which are adequate with regard to essential nutrients. The factors also improve feed efficiency in some cases.

The unidentified factors also increase production rate and hatchability.

The unidentified factors are not identical to vitamin B<sub>12</sub>, and are both organic and inorganic in nature.

On the basis of the similarity, these factors can be studied in five groups both for growth and/or production and hatchability as:

- 1. "Whey factor."
- 2. "Fish factor."
- 3. "Alfalfa or forage juice factor."
- 4. Unclassified factor(s).
- 5. Inorganic factor.

The above grouping is only arbitrary since most of the feed ingredients contain one or more factors that are present in other ingredients or substances. One substance may contain two different factors or one factor may be contained in two or more different substances.

At least two unidentified factors are necessary to obtain optimum growth rate in chicks. Better growth is obtained with the addition of two or more sources of unidentified factors than that with higher levels of each.

The activity of the unidentified factors is influenced by the type of the basal diets used, a greater increase in growth being obtained with proteins of animal source than with those of vegetable origin.

A few reports have indicated the energy level of the ration fed has an influence on the response of chicks to the unidentified factors.

Unidentified factors can be studied in a better way using purified diets than using practical rations. Furified diets have been developed for this purpose.

In general it can be stated the effect of unidentified factors with regard to egg production and hatchability is not as striking as the effect on growth.

Much further work has to be undertaken before the exact nature of these unidentified factors can be established.

Substances containing unidentified factors can be used to supplement practical rations to promote growth, and increase egg production and hatchability. Thus they play an important part in poultry nutrition and in the poultry industry at large.