THE VALUE OF CORN FERMENTATION SOLUBLES IN POULTRY NUTRITION

By

SIMON TSAI-LI TSANG

AN ABSTRACT

Submitted to the School of Advanced Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Poultry Science
Institute of Nutrition

1959

Approved: Philip J. Schaible

ABSTRACT

Evidence is presented to indicate that CFS Concentrate #3 contains unidentified growth factor(s). The growth-promoting effect of this factor(s) is modified by sexes and breeds of the birds, the origin of dietary fat, productive energy value of the ration and prolonged storage.

The addition of CFS Concentrate #3 to an all-vegetable-type ration significantly improved chick growth rate. Since the basal ration was nutritively adequate, this growth-promoting effect is used as the indication for the presence of unidentified growth factor(s) in CFS Concentrate #3. The similar effect of CFS Concentrate #3 and dried distillers solubles in stimulating chick growth suggests that they may contain the same unidentified growth factor(s). No growth response was detected when fish meal or dried brewers yeast was used in the basal ration. The chicks were not depleted for any growth factor prior to the experiment which, in part, explains the failure of the two additives to stimulate chick growth under these conditions.

Increasing productive energy from 996 to 1085 Calories per pound of ration, while the C/P ratio was kept at about 50, improved feed efficiency. With the higher caloric level, supplemental CFS concentrate #3 stimulated the growth rate of chicks. Thus, diets containing over 1000 Calories of productive energy per pound of feed are desirable if CFS Concentrate #3 or dried distillers solubles is to exert its best effect in stimulating growth of meat-type chicks.

It was effective in the presence of dietary antibiotic. However, prolonged storage of the product reduced its growth-promoting value.

White Leghorn, male birds benefited more from supplemental CFS Concentrate #3 than straight-run chicks of the same breed. Increasing the level of refined cottonseed oil from three to six per cent of the ration decreased growth rate of White Leghorn, male chicks. The effect was overcame by the addition of CFS Concentrate #3. When refined cottonseed oil was added to the diet of meat-type birds, they did not respond to CFS Concentrate #3.

The nutrients other than the unidentified growth factor(s) supplied by CFS Concentrate #3 were also utilized by chicks.

THE VALUE OF CORN FERMENTATION SOLUBLES

IN POULTRY NUTRITION

By

SIMON TSAI-LI TSANG

A THESIS

Submitted to the School of Advanced Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Poultry Science
Institute of Nutrition

1959

ACKNOWLEDGEMENTS

The author wishes to express the sincere thanks to Dr. Philip J. Schaible for his constant personal interest, motivation and guidance throughout this research.

Sincere appreciation is due to Dr. Howard C. Zindel for the many courtesies extended, and for the constructive review of this thesis. Special thanks are due Michigan State University and Clinton Corn Processing Company for financing this research through an Assistanship and a grant-in-aid.

Special thanks are due to Dr. Erwin J. Bennie for his assistance in certain chemical analyses, Dr. Robert J. Evans and Selma L. Bandemer for their help in the determination of sulfur and to Dr. Richard U. Byerrum for his constructive review of this thesis.

The author is also indebted to his fellow graduate students, Charles W. Pope, Robert H. Roberson, Hugh F. Travis, William K. Warden and J. B. Ward for their assistance and many helpful suggestions.

CONTENTS

Page

INTRODUCTION

Corn fermentation solubles is a by-product of the corn wet-milling industry. Shelled corn is the starting material and the products made from it include various grades of corn syrups, corn starches, dextrins, crude and refined corn sugar (dextrose), refined edible oil, lactic products and livestock feeds, including corn fermentation solubles.

The first operation is steeping or soaking the corn with water which softens the kernel and removes the soluble material. Five to seven gallons of water are required for every bushel of corn. Sulfur dioxide is added to the water to control fermentation and prevent putrefaction. This assists also in the extraction of the soluble compounds. During the process water is separated and reused again in steeping.

Finally, the liquid is withdrawn and concentrated to a solids' content of approximately 50 per cent. This concentrate, crude corn steep liquor, may then either be sold as it is, or combined with corn gluten feed to be marketed for use in livestock feeds.

The technology of this process is discussed in detail by Kerr (1950), Brautecht (1953), and Radley (1954). A general flowsheet of the corn wet-milling process taken from the report made by Liggett and Koffler (1948) is presented in Figure 1.

Corn steep liquor has been used for the production of penicillin since 1942 (Moyer and Coghil, 1946). More recently, it has been used also in poultry feeds, in liquid form or spray-dried on other

GENERAL FLOWSHEET

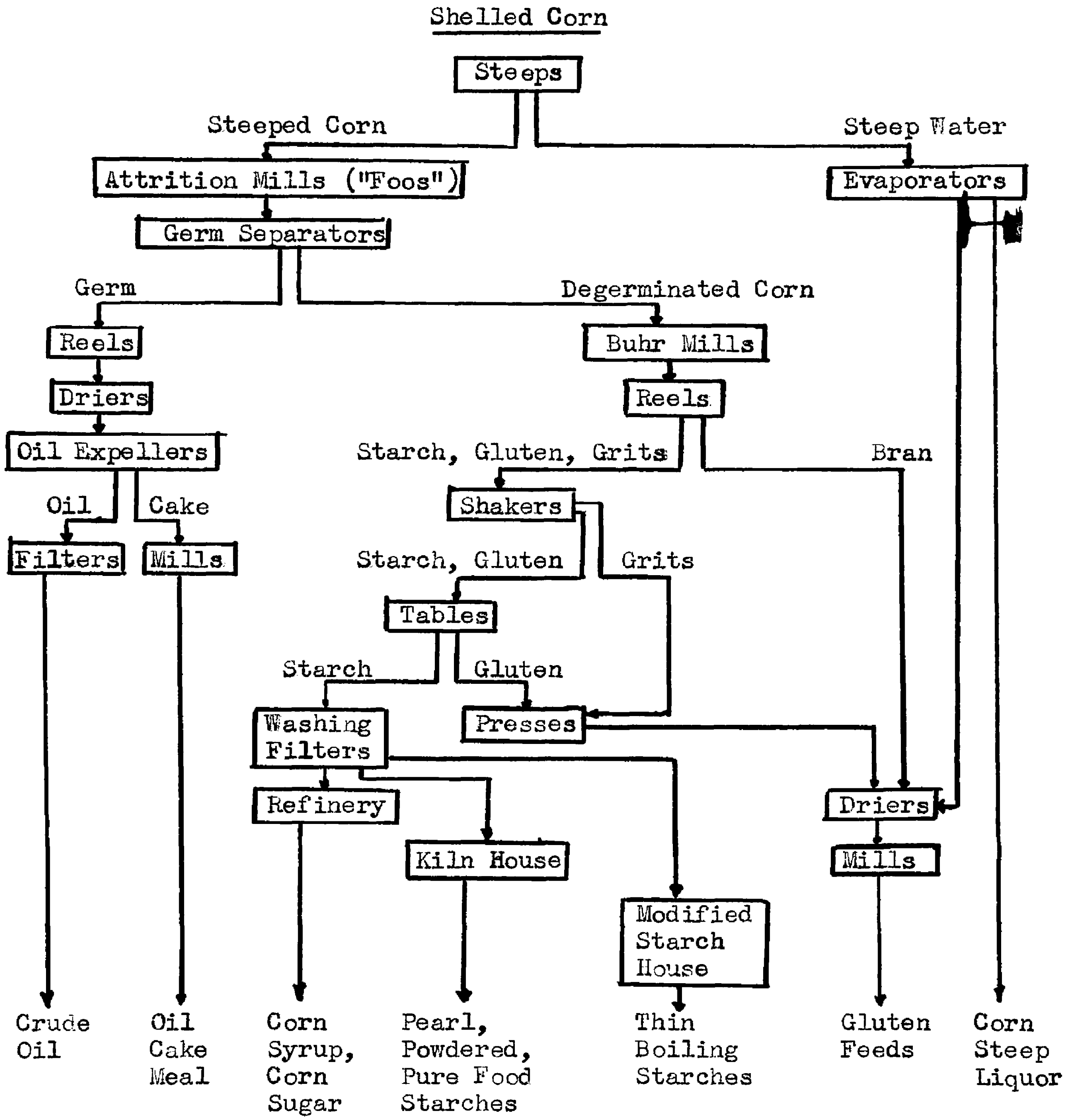

Fig. 1. General flowsheet of the corn wet-milling process. The flow of water is counter-current to the flow of materials as shown in the diagram. To maintain the water balance in the plant, water may be in process and storage for as long as two weeks. The water in the evaporator may come from any stage of the process. (Liggett and Koffler, 1948)

corn products. When steep liquor is dried on corn gluten feed, it is known as corn fermentation solubles. When supplemental vitamins are added, the product is called CFS Concentrate #3 by Clinton Corn Processing Company.

The experiments described herein were designed to evaluate CFS Concentrate #3 (CFS from hereon) in poultry nutrition.

LITERATURE REVIEW

A. The need of unidentified growth factors.

The need for certain unidentified growth factors (UGF Hereafter) by the chick is indicated by numerous reports. Carlson et al. (1949) found that the chick requires two factors, contained in fish meal and brewers dried yeast, respectively. Menge and co-workers (1952) reported that liver fractions, "L" and Biopar "C", contain the same UGF as brewers dried yeast and this differs from that in dried whey or whey products.

Fuller et al. (1952) found the chick required two UGF, one in dried whey and the other in fish solubles. Couch et al. (1952) also demonstrated that chicks, fed on an all-vegetable ration, need two UGF--one in fish meal, the other in dried distillers solubles and whey products. According to Camp et al. (1955 and 1957) an antibiotic fermentation product and fish solubles contain one factor, whereas corn steep liquor and dried whey supply another.

Norris (1954) suggested the existence of three distinct UGF--in dried distillers solubles, dried whey product or grass juice, and fish solubles. Fisher and co-workers (1954) showed that alfalfa meal, defatted whole liver, and dried whey contribute three distinct factors toward the rapid growth of chicks. According to Scott (1955) the factor contained in dried distillers solubles is different from those in alfalfa meal, whey, liver powder, and fish meal. Tamimie (1955) stated that the organic factor of fish meal is identical to the "liver" factor. Couch et al. (1952) reported that dried whey product and dried distillers solubles contain the same factor.

Three distinct UGF were recognized by the Cornell group (Norris, 1954) and four by the Illinois group (Fisher et al., 1954; Scott, 1955 and Tamimie, 1955). Discrepancies as to the number of UGF may be explained in part by the fact that conditions modify the chick response to different sources of UGF.

B. Conditions modify response to different sources of unidentified growth factors.

The influence of certain conditions on the chick response to UGF has been noticed by several workers. For example, Combs et al. (1954) postulated that differences in body stores, intestinal flora, genetic background, sex, composition of diets, as well as interrelationships among UGF modify chick response to such factors. Petersen et al. (1955) made similar suggestions. These are borne out by the findings of many other researchers.

a) Body stores or carry-over effect:

Menge et al. (1952) found that chicks from depleted hens respond to the factor supplied by a liver fraction or brewers dried yeast, whereas progeny of non-depleted hens do not. Similarly, Johnson (1953) noted that chicks from depleted hens reacted to the dietary addition of fish solubles, whereas those from non-depleted hens did not.

According to Kohler and Graham (1952) the response of the chick to factors contained in forage juice is dependent upon the make-up of the maternal diet. March et al. (1955) also stated that the factor present in dehydrated green feed is carried over from dam to chick.

d) Sex:

Arscott and Combs (1955) reported that female chicks respond to liver products or fish solubles to a greater extent than males. On the other hand, Lillie et al. (1953) found that male chicks are more responsive to the addition of fish meal.

c) Genetic background:

Researches by Lillie et al. (1958) showed that differences in breeds and systems of mating affect the carry-over of the UGF of fish solubles.

d) Composition of diets:

Barnett and Bird (1955) suggested that the change in intestinal flora due to dietary antibiotics alters the response to UGF. High levels of vitamins, as well as antibiotics, in the diet were found to spare the UGF requirement of chick (Combs et al., 1954 and Arscott and Combs, 1955).

Type of carbohydrate in the ration also influences the chick response to UGF according to Hills et al. (1953) and Arscott and Combs (1955).

Rasmussen and associates (1957) found that the value of UGF in various crude sources were more fully expressed if they were used in a ration containing a productive energy of 1077 Calories per pound of feed.

e) Interrelationships:

March et al. (1955) found out that response of chicks to either of the factors contained in dehydrated green feed or herring meal, is dependent to the presence of the other in the ration.

f) Multiplicity:

According to Combs et al. (1954) and Rasmussen et al. (1957) most of the crude UGF sources contain more than one factor. Condensed fish solubles contain both of the factors present in liver fraction and dehydrated alfalfa meal (Arscott and Combsl 1955). Dried distillers solubles contain two growth factors--"Vitamin B_{13}" and one similar to that in liver residue (Rasmussen and co-workers, 1954).

g) Variability in the potency:

Couch et al. (1954) have pointed out the existence of variations in growth factor potency among samples of different sources of UGF. Recently, Reid et al. (1958) stated that the potency of UGF in dried distillers solubles decreases during storage.

h) Evironmental microbial population:

Barnett and Bird (1956) discussed the difficulties encountered in the study of UGF in poultry nutrition. Chick responses varied from year to year over a three and half year period, due to changes in the environmental microbial population.

i) Uncertain conditions:

Camp et al. (1957) found that the incorporation of fish solubles, dried whey or corn steep liquor in an all-vegetable ration promoted chick growth in two of three experiments. They stated "chicks sometimes fail to respond to sources of unknown growth factors." Waibel et al. (1955) also referred to "the well known, but usually unpublished, difficulties in studying unidentified chick growth factors." Since negative results are frequently not published, the complete picture cannot always be ascertained.

C. The organic and inorganic natures of unidentified growth factors.

It has been found that part of the growth-promoting effect of UGF is related to the mineral content of the crude sources. The nature, both organic and inorganic, of five sources of UGF has been researched by Morrison (1955), Norris (1955), and Morrison et al. (1955 and 1956a and b). Tamimie (1955) stated that fish meal contains two UGF--one organic, the other mineral. Couch et al. (1955), Dannenburg et al. (1955), Reid et al. (1956) and Camp et al. (1956) reported that the ash of dried distillers solubles or fish solubles stimulates chick growth to the extent of about one-half that of the intact material.

On the other hand, Scott et al. (1955) did not get a growth response with the ash of dried distillers solubles whereas with the intact material they did. Briggs (1956) also failed to obtain a response by adding the ash of crude sources of UGF to a purified diet. He was critical of research workers who failed to supply levels of minerals recommended by the National Research Council (1954, NRC hereafter) in their chick rations.

D. Recent knowledge on the mineral requirements of growing chick:

Since the publication of the NRC nutrient requirements for poultry, further researches have been conducted with respect to the levels of certain minerals which give optimum results, as well as on the possible need for other trace minerals. Some of the latter have been found partially responsible for the growth-promoting effect of the ash from certain sources of UGF.

a) Potassium:

Burns et al. (1953) found that the chick requires 0.23 per cent potassium in the ration. However, 0.40 per cent gave maximum growth. According to Leach and Norris (1958) the potassium requirement varied from 0.25 to 0.32 per cent of the diet, depending upon protein and energy levels. These levels used were higher than that recommended by the NRC.

b) Zinc:

Roberson and Schaible (1958a) employed a semi-synthetic diet containing "Drackett" protein (isolated soy protein) and plastic-coated or glass equipment to show that chicks require zinc as a nutrient for normal growth, feather development, health and efficient utilization of feed. O'Dell and Savage (1957) obtained a response from supplemental zinc with "Drackett" protein but not with casein in the diet. According to Norris et al. (1958) zinc naturally present in "Drackett" protein is not utilized by the chicks whereas that in casein is. Roberson and Schaible (1958) as well as Norris and associates (1958) noted that chicks require zinc supplementation only if purified ingredients are used and certain protective measures are taken to prevent contamination. According to the former, the zinc requirement of growing chicks is 20 ppm which was confirmed by the latter workers.

O'Dell and Savage (1957) found that zinc was able to replace part of the mineral supplied by dried distillers solubles and Norris et al. (1958) stated that the zinc and potassium contents in the ash of UGF supplements do not fully explain their growth-promoting properties. Reid et al. (1958) also stated that the growth-promoting effect of dried corn distillers solubles cannot be entirely accounted for by its ash content.

c) Molybdenum:

Reid et al. (1956) and Kurnick et al. (1957) employed a diet containing "Drackett" protein and reported that chicks require molybdenum as a nutrient. The growth-promoting effect of supplemental molybdenum at 0.0216 ppm was found equivalent to that of the ash of dried distillers solubles (Reid et al., 1956) although intact product promoted a faster growth rate. When molybdenum was supplied at 0.03 ppm, growth rate almost equaled that of 3 per cent intact dried distillers solubles (Kurnich et al., 1957).

Higgins et al. (1956), and Leach and Norris (1957) produced molybdenum deficiency among chicks fed casein diets only after the addition of tungsten as an antagonist. According to Norris et al. (1958) the failure to obtain a response to supplemental molybdenum was due to the availability of this element in casein and most of the molybdenum of "Drackett" protein is not utilized by chicks. The chick's requirement for molybdenum without the antigonist--tungsten--was suggested as not more than 0.24 mg/kg of diet.

d) Selenium:

Schwarz et al. (1957) identified selenium as an integral part of "Factor 3" which was found highly effective against exudative diathesis in chicks. To produce this syndrome a torula yeast diet was employed (Schwarz et al., 1957; Scott et al., 1957 and Patterson et al. 1957). Nesheim and Scott (1957) reported that 0.1 ppm of selenium prevented the development of exudative diathesis and stimulated growth in chicks. However, when casein was used as the main source of protein in the diet, the addition of selenium did not increase the rate of chick growth (Norris et al., 1958). Since natural feedstuffs generally contain ample selenium for growth (Nesheim and Scott, 1957) and this element becomes toxic at ten ppm level (Carlson and Leitis, 1957) it is doubtful that any benefit will result by adding selenium to practical rations.

e) Bromine:

According to Bosshardt et al. (1956) the incorporation of eight or 15 ppm of bromine in a semi-purified diet promoted chick growth in three out of four experiments. Norris et al. (1957) reported that ten to 20 ppm of bromine improved chick growth slightly in two experiments; in two others, a slightly depressed growth was observed. Since bromine is plentiful in natural feed ingredients, especially salt (Ucko, 1936), whether or not chicks require supplemental bromine is still uncertain.

f) Sulfur:

The ability of ruminents to utilize inorganic sulfur has long been known. More recently, Hale and Garrigus (1953) reported that yearling wethers use elemental sulfur for cystine synthesis in the wool. To prove this, radioactive sulfur was administered as elemental sulfur or sodium sulfate. Albert et al. (1955) and Stark et al. (1953) found the inclusion of 0.2 per cent inorganic sulfur in diets supplemented with urea, promoted lamb growth. Employing an artificial rumen, Hunt et al. (1954) noted that elemental sulfur or sodium sulfate increased the micro-synthesis of riboflavin and vitamin B_{12}, and the utilization of urea. The latter was more effective. The increased synthesis of vitamins due to sulfur was attributed to the need of the microorganisms in the rumen for this mineral.

The first report on the effect of sulfur in poultry feed was made by Philips and co-workers (1921). Addition of elemental sulfur to the feed and copper sulfate to the drinking water caused a 19-per-cent growth increase over the basal at the age of eight weeks. The level of sulfur was calculated to be 1.6 per cent of the ration. The basal ration contained no added mineral except 2.44 per cent ash from an unknown source. The growth rate of all the birds was considerably lower than present-day standards.

Gordon and Sizer (1955) fed chicks a synthetic diet and found that the addition of 0.5 per cent sodium sulfate, equivalent to 0.11 per cent sulfur, showed no effect on their growth rates. Some of the minerals in this basal ration were added in sulfate forms which supplied about 0.07 per cent sulfur by calculation. When these minerals were replaced with equimolar levels of the oxide or chloride form, the addition of sodium sulfate improved both the growth rate and feed efficiency. Machlin (1955) employed a similar diet and reported that 0.5 per cent sodium sulfate gave a growth response of five to 45 per cent over the birds fed on the basal ration. It was interpretated from this that chicks have a physiological requirement for sulfate per se.

By using labeled sulfate, Machlin and associates (1952 and 1953) also demonstrated that hens can utilize inorganic sulfur for the synthesis of cystine in the eggs.

In the chick embryo, inorganic sulfur administered in the form of $H_2S^{35}O_4$ was fixed into taurine and a number of unidentified substances--but not into cystine, methionine or glutathione (Lowe and Roberts, 1955). Similar results were noted with $Na_2S^{35}O_4$ by Machlin et al. (1955). The physiological significance of taurine is still not established.

Inorganic sulfur was utilized mostly by growing chicks for the synthesis of feather cystine (Machlin et al., 1954) and protein-bound sulfate (Machlin, 1955).

There was evidence presented in the early years to show that poultry cannot utilize dietary urea (Ackerson et al., 1940; Bice and Dean, 1942). Later, Slinger et al. (1952) incorporated graded levels of urea in diets suboptimal in protein and determined that chicks were unable to utilize it for growth. In the absence of penicillin high levels of urea decreased the feed efficiency. This effect was not observed in the presence of this antibiotic. However, urea added up to four per cent of the diet showed little effect on chick growth in the presence or absence of penicillin.

More recently, Sullivan and Bird (1956) reported that urea or ammonium citrate increased the final five-week weights of birds if added to a diet containing 11 per cent protein and supplemented with methionine hydroxy-analogue. Therefore, the old belief that carnivora and omnivora cannot utilize compounds such as urea and ammonium salts (Mitchell and Hamilton, 1929) is unfounded. The possession of a rumen, in which respect poultry differs from cattle, cannot be used any longer to account for the different abilities of these two species to utilize sulfur or urea.

The author is not aware of any study made to determine if urea can increase the utilization of sulfur, or vice versa, in poultry--as has been shown in ruminents. It is possible that this phenominon may be applicable to poultry.

This review of the literature shows clearly that the ability of chicks to utilize a particular feed additive--be it UGF, urea, sulfur, or other nutrients--can only be evaluated under conditions where an inadequacy in that nutrient has been created. The failure to obtain a growth response by adding a feed ingredient to a specific ration does not prove that chicks cannot utilize this ingredient. The particular nutrient(s) may already be present in the basal ration in quantities sufficient to meet the bird's requirement. An excess will not improve performance. Attention, therefore, must be carefully given to the nutritive conditions or background under which a nutritional experiment is carried out. These considerations should be kept in mind as the author describes a number of experiments.

conducted with various basal rations in an effort to ascertain the value of CFS in poultry nutrition.

EXPERIMENTAL PROCEDURE

Since 1956, twenty experiments, involving over five thousand chicks, were designed and carried out to evaluate CFS and compare it with known sources of UGF.

As the exact procedure for each experiment varied somewhat, only the general procedure common to all experiments is given here. Any deviations in methods are described with each experiment.

A. Chicks.

Chicks were either hatched in the Department of Poultry Science or purchased from the Lambright or Cobb hatcheries. The coefficient of variation for the live weight of the male birds from the two commercial sources was 8.3 and 8.4, respectively (Davidson et al., 1957).

A summary of the breeds, sexes, ages and the sources of the chicks used in these experiments is presented in Table 1.

B. Batteries and care.

All birds under the age of five weeks were reared in starting batteries with built-in electric heaters. Afterwards they were moved to growing batteries, with no heaters, until the end of the experiments. All bateries were equipped with waterers, feeders and raised screen-floors. The screens were washed and scrubbed each time before the experiment was started.

Feed and tap water were provided at all times, except in Experiment 8 when distilled water were used.

Fourteen of the experiments were carried out in a room (in livestock pavilion) with cement floor, walls and ceiling. The room was heated by hot water, equipped with ventilation fans, and regulated at 70 - 75° F. The temperature inside of each starting battery was 98 - 95° F. for the first week and reduced about 7° F. for each succeeding week until 70 - 75° F. was reached. The starting and growing batteries were manufactured by Jamesway and Company.

The other six experiments were carried out in a frame house (poultry house #500) with cement floor, plywood walls and ceiling. The starting batteries, manufactured by Petersime and Company, and growing batteries, by Oak and Company, were located in separate rooms. No supplemental heat except, of course, the built-in heaters of the starting batteries, was provided. Ventilation was by forced-air, ceiling exhausts. The heating unit inside of each starting battery was kept on when the room temperature fell below 70° F.

C. <u>Allocation of chicks</u>.

For most experiments, chicks were allocated to pens right after their arrival. For those experiments with a preliminary standardization period allocation of birds was made following this period. The term "standardization period" referred to the period during which all chicks were fed the basal ration.

Chicks used in short term experiments were weighed individually and sorted into groups according to weight. The extremely large and small chicks were discarded. The remainders were distributed into experimental pens, balanced as to individual weight. An experimental diet was randomly assigned to each pen. For long term experiments none of the chicks were discarded.

Since a comprehensive performance test indicated the existence of significant differences among the starting batteries (poultry house #500) allocation was made in such a way so that one of each treatment was contained per battery.

If the experiment continued beyond five weeks birds were weighed individually, or by the lot, and each lot was transferred randomly to the growing batteries where they remained until the end of the experiment.

A summary of the duration of each experiment and the replication for each treatment is presented in Table 1.

D. Rations.

As the identities of UGF are unknown, the technic generally employed to detect the existence of such factors involves observations to find out if a growth response results from the incorporation of crude sources, or their ashes, in a basal ration. Therefore, a knowledge of the nutrient contents of the basal rations is exceedingly important.

a) Nature and purpose of the basal rations:

A review of literature shows that the growth-promoting effect of UGF is not necessarily dependent on the use of synthetic diets; under certain conditions it can be detected with diets of natural ingredients. Since CFS is manufactured for commercial use and certain vitamins are added to assure guaranteed levels (Table 2), it was decided to use a basal ration of natural feed ingredients.

To assure the adequacy of the basal rations, two criteria were set up. First, the rations must contain all known nutrients at

TABLE 1

THE CHICKS, DURATION AND REPLICATIONS OF EACH EXPERIMENT

	Chicks				Duration of experiment		Replications	
Expt.	Breed	Sex	Age	Source	Standardization	Experimental	Number	Chicks/group
1	W.P.R.	Male	1 day	Cobb	2 day	8 week	4	10
2*	W. L.	Str-run	2 day	M.S.U.	1 week	2 week	1	15
3*	W. L.	Male	2 day	M.S.U.	Group A-6 day	2 week	1	15
					Group B -----	20 day	2	15
					Group C -----	20 day	1	15
4*	W.P.R.	Male	1 day	Lambright	8 day	8 week	3	10
5*	W.P.R.	Str-run	1 day	M.S.U.	1 week	2 week	1	10
6*	W.P.R.	Str-run	1 day	M.S.U.	1 week	2 week	1	10
7	W.P.R.	Male	1 day	Cobb	---	8 week	4	10
8*	W.P.R.	Male	1 day	Lambright	---	4 week	3	10
9	W.P.R.	Male	1 day	Cobb	---	8 week	4	10
10	W.P.R.	Male	1 day	Cobb	---	8 week	4	10
11*	W.P.R.	Male	1 day	M.S.U.	1 week	2 week	1	15
	W. L.	Male	1 day	M.S.U.	1 week	2 week	1	15
12*	W.P.R.	Male	1 day	M.S.U.	1 week	2 week	1	15
13*	W. L.	Str-run	0 day	M.S.U.	1 week	2 week	1	10
14*	W.P.R.	Str-run	1 day	M.S.U.	1 week	2 week	1	10
15*	W.P.R.	Str-run	1 day	M.S.U.	1 week	2 week	1	10
16*	W.P.R.	Male	5 week	Exp. 8	---	4 week	2	10
17*	W.P.R.	Male	1 day	Lambright	1 day	8 week	2	10
18*	W.P.R.	Male	1 day	Lambright	1 week	4 week	3	14
19	W.P.R.	Male	1 day	Cobb	---	8 week	4	10
20	W.P.R.	Male	1 day	Cobb	---	8 week	3	10

* Note Birds of experiments with asterisk (*) were reared in livestock pavilion, others in poultry house 500.

levels equal to, or in excess of, those recommended by the NRC, or more recent publications. It was recognized that the nutrient contents of feedstuffs vary with soil conditions, seasons, etc., so any published data apply only to the particular samples analyzed. Furthermore, certain nutrient components of dehulled soybean oil meal, used in all the experiments, are not known. Secondly, a high rate of growth of young chicks was considered a good reflection of their nutritional regime. The basal rations must, therefore, support chick growth to around three pounds in live weight in about eight weeks, which is satisfactory by present standards. Under these conditions, any increased body weight due to the incorporation of a product in a ration is credited to the UGF contained therein.

An all vegetable-type of basal ration was mainly used. It contained ground yellow corn and dehulled soybean oil meal as the two protein sources.

b) Values used in calculating the nutrient levels of rations:

Except for energy values the nutrient contents of different feedstuffs, given in the "Feedstuffs Analysis Table" (Hubbell, 1957) were used in computations. The energy values, as presented in Table 3, were taken or calculated from the reports by Fraps (1946), Hill and Renner (1957), and Titus (1957) with the exception of cottenseed and corn oils. The productive energy values of these products according to Fraps (1946) were 218 and 210 therms per 100 pounds, respectively. Hill et al. (1954) claimed that these values were too low, and proposed 2900 Calories per pound of fat. Titus (1957)

TABLE 2

THE ANALYSIS OF CFS

Item	%	Amt./lb	% Protein
Crude protein, minimum*	25.0		
Crude fat, minimum*	1.0		
Crude fiber, maximum*	9.0		
N.F.E., minimum*	40.0		
Lactic acid, minimum*	4.0		
Vitamin K, Units		227	
Thiamin, mg		1.5	
Riboflavin, mg*		10	
Pantothenic acid, mg*		20	
Niacin, mg*		100	
Pyridoxine, mg		6.7	
Choline, mg (minimum)*		1000	
Folic acid, mg		0.6	
Biotin, mg		0.2	
Inositol, mg		2800	
Arginine			4.29
Histidine			3.02
Isoleucine			3.54
Leucine			10.23
Lysine			3.24
Methionine			3.20
Phenylalanine			3.80
Threonine			3.54
Tryptophane			0.70
Valine			5.51

* Levels guaranteed by the manufacturer. All the values were supplied by Clinton Corn Processing Company, Clinton, Iowa.

(1957) calculated the productive energy of vegetable oil with the aid of Fraps' factors (1946) and arrived a value of 2878 Calories per pound. Donaldson et al. (1956) used 2500 Calories for animal grease. Due to these differences, an average of the values reported by Hill, Donaldson et al. and Titus, namely 2760 Calories per pound of fat, was used.

The metabolizable energy of CFS was arrived at by multiplying the "percentage multipliers" for corn products (Titus, 1957) by the corresponding organic component supplied by CFS (Table 2) as follows:

	CFS	"Percentage Multipliers"	Calories/pound Metabolizable Energy
Protein, %	25.0	15.77	394.25
Ether extract, %	1.00	36.36	36.36
N.F.E., %	40.0	17.53	701.20
Crude fiber, %	9.0	-0.31	-2.79
		Total	1129.02

The trace mineral contents of corn and soybean oil meal were taken from various publications and are summarized in Table 4.

c) Statistics:

The data were subjected to statistical treatment for the analysis of variance (Snedecor, 1950). When a significant difference was detected, a comparison of the differences due to treatments were then determined by Duncan's "Multiple Range and Multiple F Test" (1955). In this test straight lines are drawn under certain mean values. Those means not underlined by the same continuous line are

TABLE 3

THE PRODUCTIVE AND METABOLIZABLE ENERGY VALUES USED IN THIS REPORT.

Feedstuffs	Calories/pound	
	Productive energy	Metabolizable energy
Ground yellow corn	1145[1]	1360[2]
Dehulled soybean oil meal	650[1]	999[2]
Menhaden fish meal	920[1]	921[2]
Dried distillers solubles	1020[1]	1170[2]
Corn oil meal	--	1172[3]
CFS Concentrate No. 3	--	1129[3]
Cottonseed oil	2740[4]	4000[2]
Animal tallow	2760[4]	3300[2]
Corn oil	2760[4]	4000[2]

1 Fraps (1946)
2 Hill and Renner (1957)
3 Titus (1957)
4 See text

statistically different at the confidence level indicated.

TABLE 4

THE TRACE MINERAL CONTENTS OF CORN AND SOYBEAN OIL MEAL, AND THE REQUIREMENTS BY CHICKS

Trace mineral	Corn	Soybean oil meal	Requirement % or unit of the ration
K, %	0.34[1]	2.00[1]	0.25 - 0.32[1]
Co, mg/lb	0.10[2]	?	?
Cu, mg/lb	0.9[2]	6.8[2]	0.9[2]
Fe, mg/lb	9.08[2]	0.01[3]	9[2]
Mg, mg/lb	454[4]	1543.6[4]	220[2]
Mn, mg/lb	2.22[5]	13.6[5]	25[2]
Mo, ppm	?	?	0.24[6]
Se, ppm	0.60[7]	0.03[7]	0.1 - 0.2[8]
Zn, ppm	23.4[9]	66[9]	28 - 33[9]

1 Leach and Norris (1958)
2 National Research Council (1954)
3 Woodruff and Klass (1938)
4 Morrison (1950)
5 Schaible et al. (1938)
6 Norris et al. (1958)
7 Nesheim and Scott (1957)
8 Scott (1958)
9 Norris and Zeigler (1958)

RESULTS AND DISCUSSIONS

A. Evidence indicating the presence of unidentified growth factor(s) in CFS Concentrate #3.

EXPERIMENT 1

This experiment was designed to compare the effect of CFS with that of known sources of UGF on chick performance.

The all-vegetable basal diet is presented in Table 5. Test materials were added at the expense of corn and soybean oil meal, so that the calculated protein and metabolizable energy of all rations were equalized.

Since metabolizable energy values are more reproducible than productive energy values, according to Hill and Anderson (1955), the former was used in computing energy contents of the diets. The productive energy level of the basal ration is also given in Table 5.

The birds were fed the basal ration for two days before the eight-week test began.

Results of this experiment are presented in Table 6. Tests for variance show that the treatments did not affect growth rate at five weeks, or feed efficiencies both at five and eight weeks. They did alter the final body weight.

Statistical results (P. 31) reveal that CFS and dried distillers solubles increased body weight at eight weeks whereas fish meal or brewers dried yeast did not. No additive effect was detected when dried distillers solubles, fish meal or brewers dried yeast were also incorporated in the ration containing CFS.

The similar effect of CFS and dried distillers solubles suggests

TABLE 5

THE COMPOSITION OF BASAL RATION OF EXPERIMENT 1

Ingredients	Lbs/Cwt
Ground yellow corn	53.05
Dehulled soybean oil meal	36.66
Crude corn oil	5.98
Ground limestone	1.58
Dicalcium phosphate	2.00
Iodized salt	0.50
Manganous sulfate (70%)	0.02
Terramycin (10 gm/lb)	0.048
Vitamin Mix	0.162
Calculated values:	
Protein, %	23.00
Productive energy, Cal/lb	1011
Metabolizable energy, Cal/lb	1327

Vitamin Mix:	gm/100 lb. ration
Vitamin A (10,000 IU/gm)	29.30
Vitamin D_3 (3,000 ICU/gm)	11.70
Alpha-tocopherol acetate (20 gm/lb)	4.60
Menadione	0.06
Thiamin hydrochloride	0.14
Riboflavin	0.20
Calcium pantothenate	0.20
Niacin	0.80
Choline chloride	25.70
Vitamin B_{12} (1 mg/gm)	0.60

that they may contain the same UGF. Camp et al. (1957) found that corn steepwater solubles contains factor(s) similar to that in dried whey, and Couch et al. (1952) noted that dried whey and dried distillers solubles contain similar factor(s). Consequently, the factor in CFS should be identical to that in dried distillers solubles. This was observed in the present experiment.

This growth-promoting effect of CFS should not be confused with the possibility that it may contain certain trace minerals known to be required by chicks. For example, Schwarz et al. (1957) identified selenium as an integral part of "Factor 3" supplied by certain crude sources of UGF which prevent exudative diathesis in chicks and stimulate their growth.

The trace elements known to be required by chicks as well as those supplied by the basal ration of this experiment, are presented in Table 7. It can be clearly seen that this basal ration supplied all known trace minerals, at or above the levels suggested by various workers--except, perhaps, cobalt and molybdenum.

Davis et al. (1953) found that the addition of cobalt to a diet deficient in vitamin B_{12} promoted chick growth. However, this response was not observed with a ration containing an adequate level of this vitamin. Since six mg of vitamin B_{12} were added to each pound of the rations used in these experiment, additional cobalt was not needed.

According to Norris et al. (1958) practical chick rations contain from 2.4 to 2.8 ppm of available molybdenum, which is in excess

TABLE 6

THE COMPARISON OF CFS WITH KNOWN SOURCES OF UGFS ON CHICK PERFORMANCE*
(Experiment 1)

Sources of UGF	Av. body weight, gm		Feed/gain	
	5th week	8th week	5th week	8th week
None	615	1341	1.91	2.14
3% CFS	649	1447	1.97	2.16
3% Fish meal, menhaden (FM)	650	1380	1.86	2.11
3% Dried distillers solubles (DDS)	649	1425	1.92	2.13
3% Brewers dried yeast (BDY)	617	1368	1.91	2.14
3% CFS + 3% FM	623	1414	1.90	2.11
3% CFS + 3% DDS	627	1387	1.92	2.14
3% CFS + 3% BDY	639	1429	1.93	2.17

* Four replicates of 10 birds each were used per treatment. The average initial weight per bird in each replicate was approximately 49 grams.

of the requirement (0.24 ppm). It is apparent, therefore, that the basal ration does not need supplemental cobalt or molybdenum. Consequently, the growth promoting effect of CFS or dried distillers solubles is due to factor(s) other than these trace minerals.

The lack of response from adding dried distillers solubles to the ration containing CFS is evidence that three per cent of either ingredient supplied enough of the factor(s) required by chicks for rapid growth on this basal ration.

The failure of fish meal or brewers dried yeast to increase growth rate does not mean that these additives contain no UGF. A high level of soybean oil meal was used in the basal ration and this may have prevented fish meal from showing any growth-promoting effect. Hill and Briggs (1950) reported that soybean oil meal contains UGF, and Savage et al. (1950) showed that soybean oil meal and liver residue contain the same factor(s). At the same time, Petersen et al. (1955) found that fish meal and liver residues contain the same growth factor(s).

More recently, Reid et al. (1958) found that the extraction of soybean protein with isopropanol increased the response of chicks to fish solubles but showed little influence on the growth-promoting effect of corn dried distillers solubles. Thus, unwashed soybean protein contains the "fish factor" but not factor(s) supplied by dried distillers solubles. The basal ration used in this experiment contained more than 36 per cent dehulled soybean oil meal and, thus, may have had enough "fish factor". Under these conditions, any fur-

Statistical results for body weight, Experiment 1:

8th week

Supplement	None	BDY	FM	CFS DDS	CFS FM	DDS	CFS BDY	CFS
Mean, gm	1341	1368	1380	1387	1414	1425	1429	1447

5% level

1% level

ther addition of a carrier of such factor would not be expected to increase chick body weight.

Menge et al. (1952) reported that birds from non-depleted hens showed no response to brewers dried yeast. Since commercial chicks were used in this experiment, the hens were not depleted for such growth factor(s). No response, therefore, would be expected from the supplementary brewers dried yeast. The results substantiate such assumption.

TABLE 7

THE TRACE ELEMENTS SUPPLIED BY BASAL RATION OF EXPERIMENT I IN COMPARISON WITH CHICK REQUIREMENTS*

Trace mineral	% or unit of the ration	
	Requirement	Experiment 1
K, %	0.25 - 0.32	0.91
Co, mg/lb	--	See text
Cu, mg/lb	0.9	2.97
Fe, mg/lb	9	21
Mg, mg/lb	220	806.73
Mn, mg/lb	25	30.29
Mo, ppm	0.24	See text
Se, ppm	0.1 - 0.2	0.33
Zn, ppm	28 - 33	36.6

* See Table 4 for sources of information

B. <u>Factors affecting chick response to CFS Concentrate #3</u>.

a) <u>Sex difference</u>:

EXPERIMENT 2

This experiment was designed to study the effect of CFS on the growth rate of straight-run, White Leghorn chicks.

The composition of the basal ration is presented in Table 8. Three levels of CFS were added by replacing corn and soybean oil meal so that the protein level was equalized to that of the basal.

The results are presented in Table 9. Since the feed consumption was recorded by groups, and only one group of 15 birds was used for each treatment, the feed efficiency cannot be treated statistically. However, individual weights were taken and analyzed for variance due to treatment. The variance was found not significant. Therefore, under the experimental conditions the incorporation of CFS at levels used did not affect chick growth.

TABLE 8

THE COMPOSITION OF BASAL RATION FOR EXPERIMENTS 2 AND 3

Ingredients	Experiment No. 2	Experiment No. 3 Ration A	Experiment No. 3 Ration B
	Lbs./Cwt		
Ground yellow corn	55.40	55.61	51.99
Dehulled soybean oil meal	36.73	36.70	37.32
Refined cottonseed oil	3.00	3.00	6.00
Methionine	0.05	0.05	0.05
Ground limestone	1.60	1.60	1.60
Dicalcium phosphate	2.00	2.00	2.00
Iodized salt	0.50	0.50	0.50
Trace mineral mix	0.10	0.10	0.10
Vitamin and antibiotic mix	0.62	0.44	0.44
Calculated values:			
Protein, %	23	23	23
Productive energy, Cal/lb	956	958	1003
Metabolizable energy, Cal/lb	1240	1243	1320

Trace mineral mix	gm/100 lb ration
$MnSO_4$ (70%)	10.9769
$FeSO_4 \cdot 7H_2O$	4.5159
$CuSO_4 \cdot 5H_2O$	0.3565
$CoSO_4 \cdot 7H_2O$	0.0431
$ZnCl_2$	0.0059
Dehulled soybean oil meal (carrier)	29.4607

TABLE 8 (cont'd.)

THE COMPOSITION OF BASAL RATION FOR EXPERIMENTS 2 and 3

Vitamin and antibiotic mix	Experiment No. 2	Experiment No. 3
	gm/100 lb ration	
Vitamin A (10,000 IU/gm)	40.00	40.00
Vitamin D_3 (3,000 ICU/gm)	9.00	9.00
Alpha-tocopherol acetate (20 gm/lb)	45.36	4.60
Menadione (8 gm/lb)	--	5.68
Riboflavin	0.10	0.10
Calcium pantothenate (32 gm/lb)	28.35	28.00
Niacin	1.00	1.00
Pyridoxine hydrochloride	0.23	0.23
Choline chloride (25%)	90.00	45.00
Folic acid	0.08	0.08
Biotin	0.01	0.01
Inositol	0.01	0.01
Vitamin B_{16} (6 gm/lb)	45.00	45.00
Terramycin (10 gm/lb)	22.50	22.00

TABLE 9

THE EFFECT OF CFS ON THE PERFORMANCE OF STRAIGHT-RUN WHITE LEGHORN CHICKS*
(Experiment 2)

Treatment	Av. final weight, gm	Feed/gain
Basal	195	2.05
1.5% CFS	199	1.99
3.0% CFS	196	1.97
6.0% CFS	194	1.98

* Two-day-old, straight-run, White Leghorn chicks were subjected to one week standardization period before the two week experiment. One group of 15 birds with an initial average weight of 67 grams per bird was used per treatment.

EXPERIMENT 3

This experiment was designed for two main purposes: First, to study the effect of fat levels on chick response to CFS. Second, to find out if a standardization period is required for the detection of such a response.

Two-day-old, male, White Leghorn chicks were wingbanded and divided into three groups.

Group 1: Birds were standardized on basal ration A for six days. The composition of this basal is presented in Table 8. On the seventh day, the chicks were distributed into three lots as described. Two levels of CFS were added at the expense of corn and soybean oil meal, so that each ration contained 23 per cent protein and three per cent added fat.

Group 2: Two-day-old birds were assigned randomly to their respective treatments without a standardization period. The rations used for Group 2 were the same as for Group 1 with one higher level of CFS added.

Group 3: Birds were assigned to their respective treatments as in Group 2. The composition of basal ration B is presented in Table 8. Three levels of CFS were added at the expense of corn and soybean oil meal so that each ration contained 23 per cent protein and six per cent added fat.

The results are shown in Table 10. The variances of final body weight due to treatments for Groups 1 and 3 were significant at five and one per cent levels, respectively. This indicates clearly that

TABLE 10

THE EFFECT OF CFS ON CHICK GROWTH AND FEED EFFICIENCY AS INFLUENCED BY STANDARDIZATION PERIOD AND FAT CONTENT OF THE RATIONS (Experiment 3)

Treatment	14 days			20 days		
	Average weight, gm			Average weight, gm		
	Initial	Final	Feed/gain	Initial	Final	Feed/gain
Group 1a - Birds fed rations contained 3 percent added fat with 6 days of standardization period.						
Basal	64	155	2.13			
1.5% CFS	64	176	1.94			
3.0% CFS	64	176	2.01			
Group 2b - Birds fed rations contained 3 percent added fat without standardization period.						
Basal				33	177	1.82
1.5% CFS				33	181	1.84
3.0% CFS				33	171	1.80
6.0% CFS				33	184	1.78

continued next page

TABLE 10 (cont'd.)

THE EFFECT OF CFS ON CHICK GROWTH AND FEED EFFICIENCY AS INFLUENCED BY STANDARDIZATION PERIOD AND FAT CONTENT OF THE RATIONS (Experiment 3)

Treatment	14 days: Average weight, gm		14 days: Feed/gain	20 days: Average weight, gm		20 days: Feed/gain
	Initial	Final		Initial	Final	
Group 3a - Birds fed rations contained 6 percent added fat without standardization period						
Basal				33	125	2.33
1.5% CFS				33	163	1.74
3.0% CFS				33	171	1.80
6.0% CFS				33	173	1.75

a One lot of 15 birds per treatment were used.
b Two lots of 15 birds each per treatment were used.

both the standardization period or increased fat content of the rations improved chick response toward the supplementation of CFS. With a preliminary standardization period (Group 1) the growth response due to the addition of CFS at both levels was statistically significant at the five per cent level, whereas without a standardization period (Group 2) the response was nil. The increased growth rate by adding CFS to rations containing six per cent added fat were even more obvious which was statistically significant at one per cent level.

It is shown in Table 10 that the average final weight of basal birds in Group 2 was 177 grams whereas that of Group 3 was only 125. This indicates that the increased level of refined cottonseed oil depressed chick growth. The addition of CFS to basal ration B (Group 3) restored the birds' growth rate to that of Group 2.

A growth response was obtained from the incorporation of CFS in the basal ration of Experiment 3 (Group 1), whereas in Experiment 2 no response was detected. Both basal rations were similar except for supplemental vitamins. Straight-run chicks were used in the latter case and males in the former.

The basal ration of Experiment 2 was supplemented with 45 grams of an alpha-tocopherol acetate supplement (20 gm/lb) and 90 grams of choline chloride (25%) per 100 pounds of ration and that used in Group 1 of Experiment 3 was supplemented with about four grams of the same alpha-tocopherol acetate supplement, 45 grams of choline chloride and six grams of menadione (8 gm/lb).

The analysis of CFS (Table 2) revealed that it is a good source

The statistical results, Experiment 3.

Group 1. The mean final weight

Treatment	Control	1.5% CFS	3.0% CFS
Mean	155	176	176
5% level	———	———————	———————

Group 3. The mean final weight.

Treatment	Control	1.5% CFS	3.0% CFS	6.0% CFS
Mean	125	163	171	173
5% level	———	———————	———————	———————
1% level	———	———————	———————	———————

of vitamin K and choline, but not of vitamin E. In Experiment 2 menadione was not added to the basal ration and addition of CFS did not promote chick growth. On the other hand, the basal ration of Experiment 3 was supplemented with menadione and CFS stimulated chick growth (Group 1). No supplemental vitamin K was added to the basal ration of Experiment 18. As a result birds suffered from hemorrhagic conditions whether CFS was incorporated or not. Consequently, vitamin K content of CFS cannot explain the difference observed in Experiments 2 and 3.

The choline requirement for chicks recommended by NRC is 720 mg per pound of ration. Yellow corn contains 200 mg and dehulled soybean oil meal 1500 mg choline per pound, so the basal rations in Experiments 2 and 3 supplied 857 and 759 mg. choline per pound of ration, respectively. These levels are ample to meet the chick requirement for this vitamin. The results obtained in Experiment 6 also indicate that there is no need to add more choline to a ration containing 750 mg per pound. Obviously, the choline content of CFS is not the answer for the discrepancy observed.

Sex-differences in response to UGF are well illustrated in the review of literature. Since unsexed White Leghorn chicks were used in Experiment 2 and only male chicks of the same breed used in Experiment 3, the influence of females in the former case may account for the difference observed in chick response to CFS.

The growth depression observed with the addition of refined cottonseed oil in Experiment 3 (Group 3) cannot be interpretated as due to a deficiency of folic acid in the basal ration. March and Biely (1954) found that the addition of cottonseed or herring oils to a ration depressed chick growth while tallow increased growth. The growth-depressing action of oil was counteracted by the addition of folic acid. It was found later that such detrimental action was due to the overheating of herring oil (March and Biely, 1955). When fresh oil was added to a ration suboptimal in folic acid, no depression in growth was detected. The folic acid contents of the above basal rations was not reported. However, symptoms of folic acid deficiency were observed among birds fed the basal ration. Based upon the author's calculation the basal ration was marginal in folic acid according to NRC recommendations.

Ration B of Experiment 3 is calculated to contain 1.54 mg folic acid per pound of feed, of which 0.8 mg was supplied by the vitamin mix. This level is far above the chick requirement (Table 6). The addition of six per cent CFS supplied only 0.036 mg folic acid per pound of ration which in comparison with that already provided was almost nil. Consequently, while it cannot be assured that the cottonseed oil used in this experiment was overheated or not, it is certain that the depressed growth rate was due to the added cottonseed oil per se. Since the basal ration was not deficient in folic acid, the growth stimulation which occurred with six per cent of CFS was not due to its folic acid content. It appears, therefore, that CFS aided male White Leghorn chicks in utilizing the increased level of cottonseed oil. Further, the data indicate that, under these particular experimental conditions, male White Leghorn chicks cannot tolerate six per cent of refined cottonseed oil in the diet without reducing the growth rate.

b) Breed difference:

EXPERIMENT 4

Experiment 3 showed that an increase in dietary fat improves chick response to the addition of CFS. The present experiment was designed to compare CFS with a known source of UGF, menhaden fish meal, in a ration containing six per cent of added cottonseed oil.

The birds were standardized on basal ration for eight days, after which they were allocated, as described earlier. Three replicates of ten birds were used per treatment.

Distilled water was provided. The composition of the basal ration is given in Table 11. Two levels of fish meal or CFS was added singly or in combinations. The calculated protein and fat levels of all diets were equalized to that of the basal. When methionine was used, 0.05 pound was added to each 100 pounds of ration.

Individual initial, third and eighth week week weights were taken. The average initial weight was approximately 82 grams per bird for each pen. Group feed consumption was recorded.

The results are presented in Table 12. The analyses of variances showed that the final body weights and feed efficiencies up to eight weeks due to treatments were statistically significant at five and one per cent levels, respectively. No differences of consequence were detected at the third week.

The addition of fish meal at the 5.6 per cent level depressed chick growth ($P < 0.05$) and reduced the feed efficiency ($P < 0.01$)

TABLE 11

THE COMPOSITION OF BASAL RATION FOR EXPERIMENT 4

Ingredients	Lbs/Cwt
Ground yellow corn	52.40
Dehulled soybean oil meal	37.60
Refined cottonseed oil	6.00
Ground limestone	1.32
Dicalcium phosphate	1.88
Iodized salt	0.50
Trace mineral (Delamix)*	0.10
Vitamin mix	0.20
Calculated values:	
Protein, %	23.25
Productive energy, Cal/lb	1010
Metabolizable energy, Cal/lb	1328
Vitamin mix:	gm/100 lb ration
Vitamin A (10,000 IU/gm)	33.37
Vitamin D_3 (1,500 ICU/gm)	23.00
Alpha-tocopherol acetate (20,000 ICU/lb)	4.60
Menadione	0.20
Thiamin hydroxychloride	1.00
Riboflavin	0.30
Calcium pantothenate	0.60
Niacin	1.50
Pyridoxine hydrochloride	0.20
Choline chloride	25.00
Folic acid	0.25
Inositol	0.10
Vitamin B_{12} (1 mg/gm)	0.60

* See Table 13

TABLE 12

THE EFFECT OF CFS, FISH MEAL AND SUPPLEMENTAL METHIONINE ON CHICK PERFORMANCE.
(Experiment 4)

Treatment	Average weight, gm		Feed/gain	
	3rd week	8th week	3rd week	8th week
Basal	398	1516	1.81	2.00
2.8% Fish meal (F.M.)	418	1504	1.68	2.01
5.6% F. M.	420	1418	1.75	2.16
3.6% CFS	397	1467	1.77	1.99
7.2% CFS	412	1483	1.77	2.05
2.8% FM + 3.6% CFS	427	1507	1.71	2.04
5.6% FM + 7.2% CFS	426	1477	1.74	2.08
Basal + 0.05% methionine (ME)	411	1510	1.78	1.99
5.6% FM + 0.05% ME	427	1453	1.79	2.13
7.2% CFS + 0.05% ME	404	1471	1.62	1.94

Statistical results, Experiment 4: 8th week body weight

Treat-ments	5.6% FM	5.6% FM ME	3.6% CFS	7.2% CFS ME	5.6% FM 7.2% CFS	7.2% CFS	2.8% FM	2.8% FM 3.6% CFS	Control ME	Control
Mean	1418	1453	1467	1471	1477	1483	1504	1507	1510	1516

5% level

by the end of this experiment. This detrimental effect was not detected when a lower level of fish meal was used. The addition of 0.05 per cent methionine to the diet did not improve feed efficiency. CFS at both levels, fish meal at the lower level, singly or in combinations; and supplementation of the basal ration or ration containing 7.2 per cent CFS with methionine did not alter chick performance.

Experiment 3 showed that addition of oil improved the response of the birds to CFS. CFS was found to aid chicks to utilize this added oil.

However, six per cent of cottonseed oil was also added to the rations of Experiment 4. Yet, the addition of CFS did not improve chick performance. Judged by the satisfactory growth rate of the White Plymouth Rock, male chicks, it can be said that their tolerance to cottonseed oil, or ability to utilize this refined oil, is much higher than that of White Leghorn, male chicks in Experiment 3. As a result the difference in response to CFS of these two experiments indicates a breed difference in the tolerance toward refined cottonseed oil.

Data from this experiment do not permit the author to distinguish whether the depressed chick growth was due to an imbalanced nutritive condition created by adding 5.6 per cent fish meal, or that fish meal contains a toxic factor which exerts its effect at certain levels. For instance, methionine is needed by chicks for growth, but at higher levels it depresses growth (McKittrick, 1947). Alfalfa meal contains UGF which promotes chick growth at certain low

Statistical results, Experiment 4:

8th week feed efficiency:

Treatments

	7.2% CFS ME	Control ME	3.6% CFS	Control	2.8% FM	2.8% FM 3.6% CFS	7.2% CFS	5.6% FM 7.2% CFS	5.6% FM ME	5.6% FM
Mean	1.94	1.99	1.99	2.00	2.01	2.04	2.05	2.08	2.13	2.16

5% level

1% level

levels (Fisher et al., 1954) but at higher levels, its inhibition factor (saponin) depresses growth (Peterson, 1950 a and b).

c) <u>The origin of fat</u>:

EXPERIMENT 5

This experiment was designed for two main purposes; First, to study the effect of CFS when the calculated levels of protein, riboflavin, niacin, pantothenic acid, choline, methionine and added fat were equalized. Second, to investigate the effect of supplemental sulfur on chick performance. As mentioned earlier sulfur dioxide is added to control fermentation and prevent putrefaction while the corn is being soaked with water in the wet-milling process. Consequently, a certain amount of sulfur is present in corn steep liquor.

Three levels of CFS were incorporated in the basal ration (Table 13) in such a way that all the rations contained the same calculated levels of protein, riboflavin, niacin, pantothenic acid, choline, methionine and added fat. Five graded levels of sodium sulfate (Na_2SO_4) were added to the basal ration.

The results, presented in Table 14 show no appreciable changes in body weight and feed efficiency due to treatments.

TABLE 13

THE COMPOSITION OF BASAL RATION FOR EXPERIMENT 5

Ingredients	Lbs/Cwt
Ground yellow corn	57.00
Dehulled soybean oil meal	35.31
Refined cottonseed oil	3.00
Methionine	0.08
Ground limestone	1.30
Dicalcium phosphate	2.30
Iodized salt	0.50
Trace mineral (Delamix)	0.10
Vitamin and antibiotic mix	0.41
Calculated values:	
Protein, %	23.02
Productive energy, Cal/lb	965
Metabolizable energy, Cal/lb	1248

Trace mineral (Delamix)*

Mineral	%	Present as:	At 0.1% level it supplies
Manganese	6,000	Manganous oxide	27 mg/lb of ration
Iodine	0.120	Potassium iodide	0.54 mg/lb of ration
Iron	2.0	Ferrous carbonate	9 mg/lb of ration
Copper	0.200	Copper hydroxide	0.9 mg/lb of ration
Cobalt	0.020	Cobalt carbonate	0.09 mg/lb of ration
Zinc	0.006	Zinc carbonate	0.06 mg/lb of ration
Calcium	27.000	Calcite stearate	0.027 %
Potassium**			0.00003%

continued

TABLE 13 continued

Vitamin and antibiotic mix	gm/100 lb ration
Vitamin A (10,000 IU/gm)	40.00
Vitamin D_3 (3,000 ICU/gm)	13.00
Alpha-tocopherol acetate (20,000 ICU/lb)	4.60
Menadione (8 gm/lb)	2.84
Riboflavin	0.20
Calcium pantothenate (32 gm/lb)	5.00
Niacin	1.50
Choline chloride (25%)	50.00
Vitamin B_{12} (6 mg/lb)	47.00
Terramycin (10 gm/lb)	22.00

* Briggs *et al.*, 1956

TABLE 14

THE EFFECT OF CFS AND SODIUM SULFATE ON CHICK PERFORMANCE*
(Experiment 5)

Treatment	Final Av. wt. gm	Feed/gain
Part 1 - Effect of CFS		
Basal	201	1.81
1.5% CFS	212	1.76
3.0% CFS	208	1.90
6.0% CFS	206	1.85
Part 2 - Effect of graded levels of sodium sulfate (Na_2SO_4)		
0.004% Na_2SO_4 eq 0.0009% S	200	1.91
0.008% Na_2SO_4 eq 0.0018% S	200	1.88
0.016% Na_2SO_4 eq 0.0036% S	220	1.75
0.250% Na_2SO_4 eq 0.056 % S	192	1.90
0.500% Na_2SO_4 eq 0.113 % S	220	1.78

* One-day-old, straight-run, W.P.R. chicks were subjected to 1-week standardization before the 2-week experiment. One group of 10 birds, with an average initial weight of 70 grams per bird, were used per treatment.

EXPERIMENT 6

This experiment was divided into two parts. In the first part, the basal ration and the manner in which three levels of CFS were incorporated, were the same as in Experiment 5. In the second part, graded levels of choline were added to the basal ration in order to find out if a higher level of this vitamin was required.

The results are presented in Table 15. The analysis of variance indicates no significant difference in chick weights due to treatments. Therefore, part 1 of Experiment 5 and 6 agree to each other.

The failure to step up growth by adding from 88 to 117 mg choline per pound of feed proves that the 752 mg supplied by the basal ration is ample to meet the chick's requirement for this vitamin. It further confirms the conclusion in Experiment 3 that the observed growth-promoting effect of CFS was not due to its choline content, since the basal ration of that experiment contained 759 mg choline per pound of feed.

Experiments 4, 5 and 6 show that the addition of CFS to a ration with added cottonseed oil did not affect chick performance. However, when crude corn oil or animal grease was used, as in Experiments 1, 7 and 9, CFS promoted chick growth (Table 23).

Hill et al. (1953), and Ascott and Comb (1955) found that the type of carbohydrate in the ration influences response to UGF. Possibly the origin of fat does also.

TABLE 15

THE EFFECT OF CFS AND GRADED LEVELS OF CHOLINE ON CHICK GROWTH*
(Experiment 6)

Treatment	Average final weight gm
Part 1 - Effect of CFS	
Basal	218
1.5% CFS	218
3.0% CFS	215
6.0% CFS	215
Part 2 - Effect of graded levels of choline (Basal contained 752 mg/lb)	
88 mg/lb	216
179 mg/lb	207
269 mg/lb	216
723 mg/lb	206
1177 mg/lb	214

* One-day-old, straight-run, W.P.R. chicks were subjected to a 1-week standardization before the 2-week experiment. One group of 10 birds, with an average initial weight of 69 grams per bird, were used per treatment.

d) Prolonged storage:

EXPERIMENT 7

This experiment was designed to study the effect of CFS and fish meal on chick performance when added to rations containing different levels of energy--the C/P ratio (productive energy per pound of feed/per cent of protein) being kept constant. The composition of the three basal rations is presented in Table 16.

Basal ration A: Corn oil was added to this ration to give 23 per cent protein and 1009 Calories of productive energy per pound of feed. After the birds reached the age of five weeks, the protein was adjusted to 20 per cent and productive energy to 1043 Calories.

Basal ration B: The composition of this ration is the same as ration A, except that Soka-Floc replaced Terramycin.

Basal ration C: Corn oil and Terramycin were not added to this ration. It contained 21.6 per cent protein and 935 Calories of productive energy per pound. The protein was reduced to 18.6 per cent and Calories increased to 972 after the birds reached the age of five weeks.

Although there were differences in protein and energy levels among these three basal rations, the C/P ratio was kept at approximately 43 and 52 for the periods from 0 to 5 and 5 to 8 weeks, respectively.

CFS and menhaden fish meal were added to basal ration A and B at the rate of three per cent singly or in combination. Three per

TABLE 16

THE COMPOSITION OF BASAL DIETS FOR EXPERIMENT 7

Ration	With corn oil				Without corn oil	
	A		B		C	
Ingredients	0 - 5 wk.	5 - 8 wk.	0 - 5 wk.	5 - 8 wk	0 - 5 wk.	5 - 8 wk.
	Lbs/Cwt					
Ground yellow corn	52.84	60.42	52.84	60.42	63.46	70.77
Dehulled soybean oil meal	36.70	29.37	36.70	29.37	32.13	24.82
Crude corn oil	6.00	5.80	6.00	5.80	--	--
Methionine	0.05	--	0.05	--	--	--
Terramycin (10 mg/lb)	0.048	0.048	--	--	--	--
Solka-Floc	--	--	0.048	0.048	--	--
Ground limestone	1.60	1.60	1.60	1.60	1.60	1.60
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00	2.00
Iodized salt	0.50	0.50	0.50	0.50	0.50	0.50
Trace mineral mix (Delamix)*	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin mix**	0.162	0.162	0.162	0.162	0.162	0.162
Calculated values:						
Protein, %	25	20	23	20	21.64	18.64
Prod. energy, Cal/lb	1009	1043	1009	1043	935	972
C/P ratio (Prod. energy)	43.9	52.1	43.9	52.1	43.2	52.1
Metabolizable energy, Cal/lb	1325	1347	1325	1347	1184	1210

* Table 13
** Table 5

cent CFS was also added to the basal ration C. The protein level of each experimental ration was equalized to its corresponding basal ration.

The results are presented in Table 17 and statistical results followed.

The addition of corn oil (rations 9 vs 5) increased the final weight of the chicks ($P < 0.05$) and improved their feed efficiency ($P < 0.01$). The latter was accomplished by a reduction in the amount of feed consumed per bird as well as improved protein efficiency ($P < 0.01$).

The addition of CFS to the ration containing corn oil (rations 6 vs 5) improved ($P < 0.05$) chick growth rate only to the fifth week. The particular lot of CFS used in this experiment had been stored at room temperature for more than a year with the hope that more uniformed results could be obtained by using samples from the same batch. It is quite possible that the potency of CFS decreased after the prolonged storage.

The addition of CFS to rations without corn oil (rations 9 vs 10) did not influence chick growth at the fifth and eighth week. However, it improved feed efficiency at both intervals ($P < 0.01$), and protein efficiency at the fifth week ($P < 0.01$). CFS failed to promote growth when added to this ration. The nature of the basal rations, thus, influences the response of chicks to CFS.

The addition of fish meal to the ration with corn oil (rations 7 vs 5) improved chick growth only at the fifth week ($P < 0.05$).

TABLE 17

THE EFFECT OF CFS ON CHICK PERFORMANCE AS INFLUENCED BY DIETARY FAT AND ANTIBIOTIC (Experiment 7)

Ration No.	Supplements	5th week: Av. body wt.[1] (gm)	5th week: F.E.[2]	5th week: P.E.[3]	5th week: Av. feed consumed per bird (gm)	8th week: Av. body wt.[1] (gm)	8th week: F. E.[2]	8th week: P.E.[3]	8th week: Av. feed consumed per bird (gm)
	Basal ration A. Terramycin + corn oil								
1	None	598	1.71	0.393	953	1339	2.03	0.428	2635
2	CFS	595	1.77	0.406	980	1326	2.12	0.447	2720
3	Fish meal (FM)	587	1.77	0.408	970	1314	2.10	0.444	2691
4	CFS + FM	590	1.70	0.390	931	1316	2.09	0.440	2668
	Basal ration B. Corn oil without Terramycin								
5	None	556	1.82	0.418	935	1264	2.16	0.455	2639
6	CFS	598	1.73	0.399	966	1298	2.14	0.451	2694
7	FM	596	1.82	0.418	1014	1287	2.18	0.461	2737
8	CFS + FM	610	1.88	0.431	1068	1296	2.25	0.476	2819
	Basal ration C. without Terramycin or corn oil								
9	None	535	2.38	0.516	1164	1200	2.51	0.498	2917
10	CFS	535	2.26	0.490	1119	1195	2.39	0.475	2761

1 Four replicates of 10 birds, average initial weight of 40 grams per bird, were used per treatment.
2 Feed efficiency = Feed consumed/weight gain.
3 Protein efficiency = protein consumed/weight gain.

Statistical results of Experiment 7:

1. Mean body weight (gm)

5th week

Ration No.	9	10	5	3	4	2	7	6	1	8
Supplements	C	C-CFS	B	A-FM	A-CFS FM	A-CFS	B-FM	B-CFS	A	B-CFS FM
Mean wt.	535	535	556	587	590	595	596	598	598	610
5% level										
1% level										

8th week

Ration No.	10	9	5	7	8	6	3	4	2	1
Supplements	C-CFS	C	B	B-FM	B-CFS FM	B-CFS	A-FM	A-CFS FM	A-CFS	A
Mean wt.	1195	1200	1264	1287	1296	1298	1314	1316	1326	1339
5% level										
1% level										

Statistical results of Experiment 7 (continued)

2. Feed efficiency (Feed consumed/body weight gain)

5th week

Ration No.	4	1	6	2	3	5	7	8	10	9
supplements	A-CFS FM	A	B-CFS	A-CFS	A-FM	B	B-FM	B-CFS FM	C-CFS	C
Mean	1.70	1.71	1.73	1.77	1.77	1.82	1.82	1.88	2.26	2.38
5% level										—
									—	
						—	—	—		
				—	—	—	—			
	—	—	—	—	—					
1% level										—
									—	
				—	—	—	—	—		
	—	—	—	—	—	—	—			

8th week

Ration No.	1	4	3	2	6	5	7	8	10	9
supplements	A	A-CFS FM	A-FM	A-CFS	B-CFS	B	B-FM	B-CFS FM	C-CFS	C
Mean	2.03	2.09	2.10	2.12	2.14	2.16	2.18	2.25	2.39	2.51
5% level										—
									—	
						—	—	—		
		—	—	—	—	—	—			
	—	—	—	—						
1% level										—
									—	
				—	—	—	—	—		
		—	—	—	—	—	—			
	—	—	—	—	—	—				

Statistical results of Experiment 7: (Continued)

3. Protein efficiency (Protein consumed/body weight gain)

5th week

Ration No.	4	1	6	2	3	5	7	8	10	9
Supplements	A-CFS FM	A	B-CFS	A-CFS	A-FM	B	B-FM	B-CFS FM	C-CFS	C
Mean	0.390	0.393	0.399	0.406	0.408	0.418	0.418	0.431	0.490	0.516
5% level										
1% level:										

8th week

Ration No.	1	4	3	2	6	5	7	10	8	9
Supplements	A	A-CFS FM	A-FM	A-CFS	B-CFS	B	B-FM	C-CFS FM	B-CFS	C
Mean	0.428	0.440	0.444	0.447	0.451	0.455	0.461	0.475	0.476	0.498
5% level										
1% level										

Statistical results of Experiment 7 (concluded)

4. Average feed consumed per bird (gm)

5th week

Ration No.	4	5	1	6	3	2	7	8	10	9
Supplements	A-CFS FM	B	A	B-CFS	C-FM	A-CFS	B-FM	B-CFS FM	C-CFS	C
Mean (gm)	931	935	953	966	970	980	1014	1068	1119	1164
5% level										
1% level										

8th week

Ration No.	1	5	4	3	6	2	7	10	8	9
Supplements	A	B	A-CFS FM	A-FM	B-CFS	A-CFS	B-FM	C-CFS	B-CFS FM	C
Mean (gm)	2634	2639	2668	2691	2694	2720	2737	2761	2819	2917
5% level										
1% level										

When fish meal was added with corn oil and CFS (rations 8 vs 6), no effect on chick performance was observed.

An improvement in weight gain as well as feed and protein efficiencies was observed when terramycin was added to the ration containing corn oil (Rations 5 vs 1)(P 0.05).

The addition of CFS or fish meal, singly or in combination, to the ration containing antibiotic (rations 2, 3, 4 vs 1) did not change chick performance.

Reid et al. (1958) found the potency of UGF in dried distillers solubles decreases during storage. Since CFS and dried distillers solubles contain similar UGF (Experiment 1), it is quite possible that the potency of CFS in present test decreased after prolonged storage. This probably accounts for the fact that the growth-promoting effect of CFS observed at the fifth week disappeared by the end of the experiment.

Combs et al. (1954) found that certain antibiotics spared the chick requirement for UGF. Therefore, the reduced potency of CFS in the present experiment was possibly masked by the presence of the antibiotic. The ability of CFS to promote chick growth on a diet containing an antibiotic was demonstrated in Experiments 1 and 9. These results confirm the idea that the potency of this particular lot of CFS used in the present experiment was lowered due to prolonged storage.

e) Productive energy value of the basal diet:

EXPERIMENT 8

It was the purpose of this experiment to compare known sources of UGF and CFS, their ashes, and their sulfur contents on chick growth.

Day-old, male, White Plymouth Rock chicks from a commercial hatchery were distributed as described previously, with three replicates of ten birds per treatment. The average initial weight per chick of each pen was 41 grams.

The composition of the basal ration is presented in Table 18. Materials were ashed by charring and then placing in a muffle furnace at 525° C. for approximately 13 hours. Their calcium, phosphate and sulfur contents were analyzed according to AOAC methods (1955) and are presented in Table 9. During charring and ashing certain amounts of sulfur might have been driven off. The sulfur content in the ashes therefore, were lower than that reported in Table 34. Three types of sulfur were added at graded levels. The calculated protein, calcium and phosphate levels of all rations were equalized to that of the basal.

Group weights were taken at the first day and the end of fourth week. The results are presented in Table 20. The statistical results are indicated by asterisks since the usual method of underling (Duncan's test, 1955) would be too long for 19 treatments.

All treatments, except two higher levels of sodium sulfate, showed no effect on chick growth at the end of four weeks. These

TABLE 18

THE COMPOSITION OF BASAL RATION FOR EXPERIMENT 8

Ingredients	Lbs/Cwt
Ground yellow corn	56.71
Dehulled soybean oil meal	36.45
Animal grease	3.00
Methionine	0.05
Ground limestone	1.31
Dicalcium phosphate	1.88
Iodized salt	0.50
Trace mineral (Delamix)*	0.10
Calculated values:	
Protein, %	23.04
Productive energy, Cal/lb	969
Metabolizable energy, Cal/lb	1227

Vitamins	Grams added to 100 lb ration
Vitamin A(250,000 IU/gm)	1.60
Vitamin D_3 (1,500 ICU/gm)	26.00
Alpha-tocopherol acetate (20,000 ICU/lb)	4.60
Menadione	0.10
Thiamine hydrochloride	0.30
Calcium pantothenate	0.50
Niacin	1.50
Pyridoxine hydrochloride	0.20
Choline chloride	10.00
Folic acid	0.10
Inositol	0.10
Vitamin B_{12} (1 mg/gm)	0.60
Total	45.90 grams

* See Table 13

TABLE 19

ASHES AND THEIR MINERAL CONTENTS OF TESTING MATERIALS USED IN EXPERIMENT 8

Item	Ash, %	Percent of ash		
		Ca	P	S*
CFS Concentrate #3	5.46	2.07	14.84	2.00
Dried distillers solubles	8.43	2.96	17.72	0.34
Fish concentrates	15.61	19.98	14.64	0.20
Vigofac	58.42	1.87	8.60	6.21

* Sulfur present in the ash as the sulfate ion.

results indicate that the basal ration did not meet the conditions for sources of UGF to exert their growth-promoting effect.

The addition of 0.013 and 0.022 per cent of sodium sulfate depressed chick growth severely ($P < 0.01$). However, chick growth was not affected by the addition of the same or higher levels of sulfur, when supplied as flowers of sulfur or calcium sulfate. Therefore, it was sodium, rather than sulfate, which caused the growth-depression. Since the depressed growth rate, due to the addition of 0.013 per cent sodium sulfate, was lower ($P < 0.01$) than 0.022 per cent, it indicates the possibility of an imbalanced relationship between sodium and potassium or other nutrients contained in the ration.

Burns and co-workers (1953) found that both sodium and potassium are toxic to chicks if one was fed in excess of the other, and this toxicity could be corrected by raising the level of the other element. This finding explains the results obtained in the present experiment.

TABLE 20

THE EFFECT OF KNOWN SOURCES OF UGFS AND CFS, THEIR ASHES AND SULFUR CONTENTS ON CHICK GROWTH

Treatment	Percent ration		Av. 4-week weight, gm
	Equivalent intact material	Sulfur supplied	
Basal			362
Ash of fish concentrate	3.3	0.001	372
Ash of CFS	2.8	0.003	355
Ash of dried distillers solubles	3.5	0.001	363
Ash of vigofac	0.14	0.005	360
Flowers of sulfur		0.001	366
Flowers of sulfur		0.003	350
Flowers of sulfur		0.005	365
Sodium sulfate		0.001	369
Sodium sulfate		0.003	130**
Sodium sulfate		0.005	186**
Calcium sulfate		0.001	370
Calcium sulfate		0.003	374
Calcium sulfate		0.005	372
Calcium sulfate		0.010	356
Fish concentrate	3.3		384
CFS	2.8		359
Dried distillers solubles	3.5		352
Vigofac	0.14		371

** Significant at 1% level from all other groups.

EXPERIMENT 9

It was the purpose of this experiment to study the influence of the productive energy value of the basal ration and supplemental trace minerals on the growth-promoting effect of CFS.

Two basal rations were formulated (Table 21):

Basal A: This ration contained 20 per cent protein, 1.2 per cent calcium and 996 Calories of productive energy per pound of feed.

Basal B: Protein and productive energy levels of this ration were raised up to 22 per cent and 1085 Calories per pound of ration, respectively. The calcium content was the same as in A.

The C/P ratio, based on productive energy, was 50 for basal A and 49 for B. When metabolizable energy values were used, all contained the same C/P ratio of 62.

Delamix, a commercial mineral supplement containing trace elements other than manganese (Table 13), was employed to replace the manganous sulfate, Solk-Floc and 0.07 per cent of ground limestone in basal rations A and B. Therefore, the protein, calcium, manganese and energy levels were equalized to the respective basal ration.

Three per cent of CFS was added to basal rations A and B, with or without supplemental trace elements. The calculated protein level of each experimental ration was equalized to its corresponding basal ration.

Individual weights were taken at the end of eight weeks. Ana-

TABLE 21

THE COMPOSITION OF BASAL RATION OF EXPERIMENT 9

Ingredients	Ration	
	A	B
	Lbs/Cwt	
Ground yellow corn	63.44	49.46
Dehulled soybean oil meal	28.83	35.30
Animal yellow grease	2.98	10.49
Ground limestone	1.67	1.67
Dicalcium phosphate	2.00	2.00
Iodized salt	0.50	0.50
Manganous sulfate (70%)	0.02	0.02
Solka-floc	0.01	0.01
Vitamin and antibiotic mix	0.55	0.55
Calculated values:		
Protein, %	20	22
Calcium, %	1.2	1.2
Productive energy, Cal/lb	996	1085
Metabolizable energy, Cal/lb	1249	1371
C/P		
(productive)	50	49
(metabolizable)	62	62

Vitamin and antibiotic mix	Unit/100 lb ration
Vitamin A(10,000 IU/gm), lb	0.06
Vitamin D_3 (3,000 ICU/gm), lb	0.03
Alpha-tocopherol acetate (20 gm/lb), gm	4.5
Menadione (crystalline), gm	0.04
Vitamin mix No. 1, lb	0.10
Choline chloride (25%), lb	0.20
Vitamin B_{12} (6 mg/lb), lb	0.10
Terramycin (10 mg/lb), lb	0.05
Total	0.55 lb

* Supplied: riboflavin 2, pantothenic acid 4, niacin 9, and choline 10 grams per pound.

lysis of variance showed that the treatments altered the final weights and feed efficiencies. The magnitude of the differences were highly significant.

Although Basal A contained less protein and energy than Basal B, these differences did not affect final weights. However, feed efficiency was improved ($P < 0.01$).

The substitution of Delamix for manganous sulfate in Basal A depressed growth rate ($P < 0.05$), but showed no effect on feed efficiency. This substution in Basal B did not affect chick growth but improved feed efficiency ($P < 0.05$). Since manganese is not the only one mineral in Delamix, the other trace elements must account for the effects observed. These data, therefore, show that the chick requirements for trace minerals, other than manganese, vary with the protein and energy contents of the ration.

Although the incorporation of CFS in Basal A showed no effect when added to Basal B it increased final weights ($P < 0.01$). These results emphasize that the plane of nutrition--levels of protein and energy--influences chick response to CFS.

CFS increased growth rate ($P < 0.05$) when added to Basal A with supplemental trace minerals. This indicates the ability of CFS to correct the unbalanced mineral relationship created by the elements, other than manganese supplied by Delamix. The increased body weight obtained by adding CFS to Basal B with supplemental trace minerals was not statistically significant. However, the addition of CFS to Basal B improved chick growth ($P < 0.01$). Yet, the substitution

TABLE 22

THE EFFECT OF CFS ON CHICK PERFORMANCE AS INFLUENCED BY THE POMPOSITIONS OF RATION (Experiment 9)

Treatment	Av. final wt. gm.	Feed/gain
A	1321	2.39
A + CFS	1314	2.32
A + trace minerals (Tm)	1253	2.30
A + CFS + Tm	1329	2.38
B	1323	2.19
B + CFS	1414	2.10
B + TM	1385	2.06
B + CFS + Tm	1428	2.03

* Four replicates of 10 birds each were used per treatment. The average initial weight per bird in each replicate was approximately 40 grams.

Statistical results for 8-week body weight, Experiment 9:

Variables	A, Tm	A CFS	A	B	A, Tm CFS	B, Tm	B CFS	B, Tm CFS
Mean	1253	1314	1321	1323	1329	1385	1414	1428
5% level								
1% level								

Statistical results for 8-week feed efficiency, Experiment 9:

Variables	B, Tm CFS	B, Tm	B CFS	B	A, Tm	A CFS	A, Tm CFS	A
	2.03	2.06	2.10	2.19	2.30	2.32	2.38	2.39
5% level								
1% level								

of Delamix for manganous sulfate in Basal B did not change the chick growth rate. Therefore, the above results indicate clearly that CFS was able to improve chick body weights, with or without complex trace mineral supplement.

The incorporation of CFS to the basal ration of Experiment 8, containing 23 per cent protein and 969 Calories of energy, did not stimulate chick growth. However, the growth-promoting effect of CFS was detected on ration containing 22 per cent protein and 1085 Calories of energy, in Experiment 9. Thus, these two experiments indicate that the energy level influences chick response to the UGF supplied by CFS. This influence was also illustrated in previous experiments (Table 23).

The question, whether it is the level of energy or fat which realy regulates chick response to CFS, naturally comes up at this point.

The mechanism through which fat exerts its effect appears to differ for different sources. Rand et al. (1957) reported that on isocaloric diets corn oil improves chick weight gain and protein retention. Therefore, other than its caloric value, corn oil appears to possess an additional intrinsic value. Baldini and Rosenberg (1957), and Vondell and Ringrose (1958) found the increased energy level by adding beef tallow or raising carbohydrate content equally improved chick growth and feed efficiency. Thus, the nutritional effect of tallow, aside from its fatty acid content, is credited entirely to its caloric value. Consequantly, beef tallow does not contain the addi-

TABLE 23

DIETARY FACTORS INFLUENCE CHICK RESPONSE TO SUPPLEMENTAL CFS

Expt. No.	Protein %	Added fat Source	Added fat %	Productive energy, Cal/lb	C/P ratio	Antibiotic	Response to CFS
1	23	C.C.O.	5.98	1011	44	+	+
4	23.25	C.S.O.	6.0	1010	43.4	-	-
5	20	C.S.O.	3.0	965	48	+	-
6	20	C.S.O.	3.0	965	48	+	-
7	23-20	C.C.O	6 - 5.8	1009 - 1043	44 - 52	+	-
	23-20	C.C.O.	6 - 5.8	1009 - 1043	44 - 52	-	+ (fifth week)
	22 - 19	---	---	935 - 972	43 - 52	-	-
8	23	A.G.	3.0	969	42	-	-
9	20	A.G.	2.98	996	50	+	-
	22	A.G.	10.49	1085	49	+	+

Note: C.C.O. = Crude corn oil; C.S.O. = Refined cottonseed oil; A. G. = Animal yellow grease.

tional value as corn oil does.

Crude corn oil was used in Experiment 1 and animal yellow grease which contains various amounts of beef tallow, was used in Experiment 9. The addition of CFS promoted chick growth in both instances. Therefore, it is energy not fat that regulates chick response to CFS.

Hill and Dansky (1950), Leong et al. (1955) and Donaldson et al. (1956) reported that the productive energy level influences the optimum protein level of the chick's diet. Sunde (1956) reported also that a change in protein level changes the optimum C/P ratio. On the other hand, Vondel and Ringrose (1958) found that chicks grow at the same rate up to 8 weeks when the C/P ratio lies within the range of 45 to 53, irrespective of the dietary protein level. At the same time, the increased energy value improves feed efficiency.

At the first glance, the performances of chicks fed the two basal rations in Experiment 9 appear to agree with the findings of Vondel and Ringrose and disagree with the other reports cited above. However, after a careful calculation it was found that the ranges of C/P ratio and productive energy values, employed by the above authors, were not the same. Sunde used two levels of protein, 20 and 28 per cent; with ranges of productive energy value from 655 to 932 Calories per pound and of C/P ratio from 23 to 47. Vondel and Ringrose used three levels of protein; 16.5, 18.5 and 22.5 per cent with productive energy value from 611 to 1190 Calories and C/P ratios from 36 to 53. The optimum C/P ratio reported by the latter falls within the upper range of that employed by the former. Therefore, it appears possible that both of these schools of thought apply. When the C/P ratio lies within the range of 45 to 53, chicks grow at the same rate, irrespective of the dietary protein level. However, when the C/P ratio lies below 45, the protein level changes the optimum ratio.

C. Replacement value of CFS Concentrate #3 for fish meal.

EXPERIMENT 10

An eight-week experiment was designed to test the replacement value of CFS for menhaden fish meal. The basal ration contained three per cent fish meal (Table 24).

Test materials replaced a corresponding amount of fish meal (Table 25), except for the last two rations where they were added at the expense of corn and soybean oil meal. Protein levels of all rations were equalized.

The results are presented in Table 23. The analyses of variance show that body weights were affected by treatments at both the fifth and eighth weeks ($P < 0.01$). At five weeks, the treatments only altered feed efficiency ($P < 0.05$).

The influence of treatments on chick performance are illustrated on pages 84 and 85. The replacement of fish meal by CFS increased chick growth at both fifth and eighth weeks ($P < 0.05$); however, its influence in improving feed efficiency was significant only at the fifth week ($P < 0.05$). Thus, at the three per cent level, CFS proved better than fish meal as a supplement to the all-vegetable basal ration. When only half of the fish meal was replaced by CFS, chick performance was not affected.

When corn oil meal replaced fish meal at the three per cent level, growth rate was reduced ($P < 0.01$), but there was no effect on feed efficiency. The substitution of 1.5 per cent fish meal by corn oil meal depressed final chick weight ($P < 0.05$). The addi-

TABLE 24

THE COMPOSITION OF BASAL RATION OF EXPERIMENT 10

Ingredients	Lbs/Cwt
Ground yellow corn	51.75
Dehulled soybean oil meal	33.29
Menhaden fish meal	3.00
Prime tallow	7.96
Ground limestone	1.58
Dicalcium phosphate	2.00
Iodized salt	0.50
Manganous sulfate (70%)	0.02
Terramycin (10 mg/lb)	0.048
Vitamin mix*	0.162
Calculated values:	
Protein, %	23.00
Productive energy, Cal./lb	1056
Metabolizable energy, Cal/lb	1327

* See Table 5

TABLE 25

THE REPLACEMENT VALUE OF CFS AND CORN OIL MEAL FOR FISH MEAL
(Experiment 10)

Variables	Av. body weight, gm		Feed/gain	
	5th week	8th week	5th week	8th week
3% Fish meal (FM)	656	1380	1.95	2.14
1.5% FM + 1.5% CFS	681	1378	1.88	2.12
3% CFS	696	1434	1.87	2.11
1.5% FM + 1.5% Corn oil meal (COM)	626	1318	1.93	2.14
3% COM	637	1306	1.96	2.14
1.5% CFS + 1.5% COM	629	1328	1.96	2.16
3% CFS + 3% COM	641	1354	1.84	2.08
3% CFS + 3% COM + 3% FM	652	1367	1.90	2.13

* Four replicates of 10 birds each were used per treatment. The average initial weight per bird in each replicate was approximately 39 grams.

Statistical results for body weight, Experiment 10

5th week

Variables	1.5% FM 1.5% COM	1.5% CFS 1.5% COM	3% COM	3% CFS 3% COM	3% CFS 3% FM 3% COM	3% FM	1.5% CFS 1.5% FM	3% CFS
Mean	626	629	637	641	652	656	681	696
5% level								
1% level								

8th week

Variables	3% COM	1.5% FM 1.5% COM	1.5 CFS 1.5% COM	3% CFS 3% COM	3% CFS 3% FM 3% COM	1.5% CFS 1.5% FM	3% FM	3% CFS
Mean	1306	1318	1328	1354	1367	1378	1380	1434
5% level								
1% level								

Statistical results for feed efficiency, Experiment 10

5th week

Variables	3% CFS 3% COM	3% CFS	1.5% CFS 1.5% FM	3% CFS 3% FM 3% COM	1.5% FM 1.5% COM	3% FM	1.5% CFS 1.5% COM	3% COM
Mean	1.84	1.87	1.88	1.90	1.93	1.95	1.96	1.96
5% level				—	—	—	—	—
			—	—	—	—		
		—	—	—	—			
	—	—	—	—				
1% level		—	—	—	—	—	—	—
	—	—	—		—	—			

tion of two levels of corn oil meal to the ration containing CFS depressed growth at fifth and eighth weeks ($P < 0.01$). When fish meal and corn oil meal were added to ration containing CFS, chick growth rates for the fifth and eighth weeks were reduced ($P < 0.01$ and $P < 0.05$, respectively).

Data from Experiment 1 indicated that the corn and soybean oil meal type of ration contains enough UGF supplied by fish meal. However, this type of ration is not sufficiently supplied with the UGF contained in CFS or dried distillers solubles. Consequently, the replacement of CFS for fish meal should increase chick performance. The results obtained in the present experiment substantiate this.

According to Draper (1944) corn oil meal is deficient in cysteine, lysine and possibly glutamic acid. Hence the replacement of corn oil meal for fish meal, or the addition of the former to ration containing CFS reduced certain critical amino acids in the ration and resulted in an upset in the amino acids balance. This probably caused chick growth to be depressed.

D. Availability of vitamins contained in CFS Concentrate #3.

EXPERIMENT 11

Composition of the basal ration, grossly deficient in vitamins, is presented in Table 26. Three levels of CFS were added at the expense of corn and Amisoy, an isolated soybean protein, so that each ration contained 23 per cent protein and three per cent added fat.

The results are presented in Table 27. Statistical results indicate that six per cent CFS promoted chick growth over the basal for both breeds of birds ($P < 0.01$). The incorporation of three per cent CFS increased chick weight for White Leghorn but not White Plymouth Rock, males ($P < 0.01$). These indicate a breed difference in the response to CFS.

The addition of CFS also improved the feed efficiency (Table 25). Among White plymouth Rock birds, when 1.5 per cent CFS were used the feed efficiency was slightly decreased. However, when three or six per cent CFS were used the feed efficiency was sharply increased.

The improvement in feed efficiency for White Leghorn chicks increased with each higher levels of CFS.

The vitamin contents of this basal ration were lower than the NRC recommendation (Table 28). Since only 0.1 gram of manganous sulfate (70%) was added per 100 pounds of ration, the basal ration was also deficient in this mineral. Such deficiencies were also reflected by the poor performances of the basal groups.

TABLE 26

THE COMPOSITION OF BASAL RATION FOR EXPERIMENT 11

Ingredients	Experiment No. 11 (Lbs/Cwt)	Experiment No. 12 (Lbs/Cwt)
Ground yellow corn	74.21	71.25
Amisoy	19.00	19.30
Refined cottonseed oil	3.00	3.00
Methionine	0.05	0.05
Mineral mix	3.719	4.20
Vitamin and antibiotic mix	0.021	2.20
Calculated protein level, %	23.	23.

Mineral mix	gm/100 lb ration 11	12
Ground limestone	598.7221	955.76
Dicalcium phosphate	908.00	696.71
Iodized salt	181.60	227.00
Manganous sulfate (70%)	0.10	14.50
$CuSO_4 \cdot 5H_2O$	0.0035	0.35
$CoSO_4 \cdot 7H_2O$	0.0004	0.04
$FeCl_3 \cdot 6H_2O$	--	13.07

Vitamin and antibiotic mix	gm/100 lb ration	
Vitamin A (10,000 IU/gm)	1.6000	36.00
Vitamin D_3 (1,500 ICU/gm)	1.3000	9.00
Alpha-tocopherol acetate (20 gm/lb)	0.2040	2.00
Riboflavin	0.0040	0.39
Calcium pantothenate	0.0080	1.37
Niacin	0.0400	3.60
Pyridoxine hydrochloride	0.0090	0.47
Choline chloride (25%)	3.6616	829.96
Folic acid	0.0036	0.08
Biotin	0.0004	0.01
Vitamin B_{12} (6 mg/lb)	1.8000	90.80
Para-aminobenzoic acid	0.0036	0.04
Terramycin (10 gm/lb)	0.8988	22.70

* Vitamin D_3 supplements 1,500 and 3,000 ICU/gm were used in Expt. 11 and 12, respectively.

TABLE 27

THE RESULTS OF EXPERIMENT 11*

Treatments	Average final wt. gm	Feed/gain
W. P. R. male		
Basal	80	3.37
1.5% CFS	84	3.48
3.0% CFS	96	2.83
6.0% CFS	124	1.81
W. L., male		
Basal	68	4.4
1.5% CFS	78	3.4
3.0% CFS	88	3.0
6.0% CFS	97	2.5

* Two-week experiment preceded by 1-week standardization period. Fifteen birds per treatment were used, with an average initial of 53 and 49 grams per bird for W. P. R. and W. L., respectively.

The statistical results, Experiment 11

The mean final weight of male W.P.R. chicks

Treatments	Control	1.5% CFS	3.0% CFS	6.0% CFS
Mean	80	84	96	124
5% level				
1% level				

The mean final weight of male W. L. chicks

Treatments	Control	1.5% CFS	3.0% CFS	6.0% CFS
Mean	68	78	88	97
5% level				
1% level				

TABLE 28

CALCULATED VITAMIN CONTENT OF BASAL RATIONS EMPLOYED IN EXPERIMENT 11 AND 12, AS COMPARED WITH NRC REQUIREMENT

Vitamins	Unit/100 lb ration		
	Experiment No.		NRC[1] requirement
	11	12	
Vitamin A, IU	16,000[2]	360,000[2]	199,200
Vitamin D, ICU	1,950[2]	27,000[2]	13,500
Alpha-tocopherol acetate, mg	135[3]	209[3]	---
Vitamin K, mg	?	?	22
Thiamin, mg	127	122	96
Riboflavin, mg	41[3]	426[3]	156
Pantothenic acid, mg	196	1,441	504
Niacin, mg	720	4,254	1,440
Pyridoxine, mg	13[4]	396[4]	156
Choline, mg	15,636[3]	199,761[3]	72,000
Folic, mg	11	83	30
Biotin, mg	1.00[4]	13[4]	5
Inositol, mg	1.00[2]	10[2]	--
Vitamin B_{12}, mg	0.03[4]	1.20[4]	0.48
Para-aminobenzoic acid, mg	4[2]	36.00[2]	--

1 National Research Council (1954). The levels listed include the margins of safety, which were 66 percent for vitaminA, 50 percent for vitamin D, and 20 percent for the water-soluble vitamins.
2 Supplied by vitamin mix only.
3 Supplied by vitamin mix and corn.
4 Supplied by vitamin mix and Amisoy.

EXPERIMENT 12

Composition of the basal ration with high levels of supplemental vitamins is presented in Table 26. The calculated vitamin contents of this ration is found in Table 28 in comparison with that of Experiment 11 and the chick requirement suggested by NRC. Three levels of CFS were added in the same manner as in Experiment 11.

The results are presented in Table 29. Upon analysis the variance due to treatments was found not significant.

The treatments as well as management in Experiments 11 and 12 were the same. Day-old, male, White Plymouth Rock chicks were also used in Experiment 11. But, the basal ration employed in Experiment 11 was deficient in vitamins, to which the incorporation of CFS promoted chick growth. While the addition of CFS to basal ration of Experiment 12, which contained higher levels of vitamins, no growth response was observed. Therefore, the results from these two experiments indicate that the vitamins supplied by CFS are available to the chicks.

TABLE 29

THE RESULTS OF EXPERIMENT 12*

Treatments	Average final weight, gm	Feed/gain
Basal	222	1.61
1.5% CFS	213	1.69
3.0% CFS	209	1.78
6.0% CFS	202	1.83

* Day-old, male, W.P.R. chicks were subjected to 1-week standardization before the 2-week experiment. Fifteen birds per treatment with an average initial weight of 63 grams per bird were used.

TABLE 30

THE EFFECT OF SODIUM SULFATE AND CALCIUM ON CHICK PERFORMANCE* (Experiment 13)

Treatment	Average final weight, gm	Feed/gain
Basal	198	1.77
0.1% Na_2SO_4 eq 0.023% S	192	1.84
0.3% Na_2SO_4 eq 0.068% S	211	1.78
0.5% Na_2SO_4 eq 0.113% S	196	1.81
0.3% Na_2SO_4 + 0.2% Ca	196	1.83

* Straight-run W.P.R. chicks were subjected to 1-week standardization, started at the day hatched, before the 2-week experiment. Ten birds per treatment with an initial average weight of 64 grams per bird were used.

E. Effect of supplemental sulfur on chick performance.

EXPERIMENT 13

This experiment was a repetition of Part 2 of Experiment 5 in testing the effect of sodium sulfate on chick performance. The basal ration was the same (Table 13) and sodium sulfate was added at 0.1, 0.3, and 0.5 per cent levels. In one other treatment 0.2 per cent of calcium in the form of ground limestone was added to ration containing 0.3 per cent sodium sulfate. Since the basal ration calculated 1.31 per cent calcium, this addition made a total of 1.51 per cent.

The results are presented in Table 30. The analysis of variance shows that the treatment did not alter chick performance. These results are in accord with the findings in Experiment 5. Sodium sulfate, added up to 0.5 per cent of the diet, did not affect chick performance.

EXPERIMENT 14

This experiment was designed to study further the effect of supplemental sulfate on chick performance. In Experiments 5 and 13 sodium sulfate was used whereas in this experiment calcium sulfate ($CaSO_4 \cdot H_2O$) was employed. The calcium level of all rations were equalized.

Composition of the basal ration is presented in Table 31. For each level of added calcium sulfate an equivalent amount of ground limestone was substracted and the amount of corn and soybean oil meal adjusted so that the calculated protein and calcium levels were equalized to the basal ration.

The results presented in Table 32 indicate that supplemental sulfur, added in the form of calcium sulfate up to 0.12 per cent of the diet, did not affect chick growth.

TABLE 31

THE COMPOSITION OF BASAL RATION FOR EXPERIMENT 14

Ingredients	Lbs/Cwt
Ground yellow corn	56.19
Dehulled soybean oil meal	36.44
Animal grease	3.00
Methionine	0.05
Ground limestone	1.30
Dicalcium phosphate	2.30
Iodized salt	0.50
Trace mineral (Delamix)*	0.10
Vitamin and antibiotic mix	0.12
<u>Calculated values:</u>	
Protein, %	23.00
Productive energy, Cal/lb	963
Metabolizable energy, Cal/lb	1227
<u>Vitamin and antibiotic mix</u>	<u>gm/100 lb ration</u>
Vitamin A (250,000 IU/gm)	1.60
Vitamin D_3 (1,500 ICU/gm)	26.00
Alpha-tocopherol acetate (20,000 ICU/lb)	4.60
Menadione (8 gm/lb)	2.84
Thiamine hydrochloride	0.30
Riboflavin	0.20
Calcium pantothenate (32 gm/lb)	5.00
Niacin	1.50
Pyridoxine hydrochloride	0.20
Choline chloride	10.80
Folic acid	0.10
Vitamin B_{12} (1 mg/gm)	0.60
Sodium procaine penicillin (500 gm/kg)	1.00

* See Table 12 for composition.

TABLE 32

THE EFFECT OF SULFUR, SUPPLIED AS CALCIUM SULFATE ON CHICK GROWTH*
(Experiment 14)

Treatment	Average final weight, gm
Basal	235
0.04% S	249
0.06% S	231
0.08% S	243
0.10% S	232
0.12% S	237

* Day-old, straight-run, W.P.R. chicks were subjected to 1-week standardization before the 2-week experiment. Ten birds per treatment, with an initial average weight of 75 grams per bird were used.

TABLE 33

THE EFFECT OF FLOWERS OF SULFUR ON CHICK GROWTH*
(Experiment 15)

Treatment	Average final weight, gm	Feed/gain
Basal	224	1.75
0.05% S	218	1.73
0.10% S	231	1.69
0.50% S	209	1.81
1.00% S	223	--
1.50% S	234	1.67

* Day-old, straight-run, W.P.R. chicks were subjected to 1-week standardization before the 2-week experiment. Ten birds per treatment, with an initial average weight of 72 grams per bird were used.

EXPERIMENT 15

This experiment was designed for the same purpose as Experiment 14. However, sulfur was added as flowers of sulfur in basal ration of Experiment 14.

The results are presented in Table 33. Upon statistical analysis, differences in final weights due to treatments were found not to be significant.

Gordon and Sizer (1955) found that the addition of 0.5 per cent sodium sulfate to a ration containing 0.07 per cent inorganic sulfur showed no effect on chick growth rate. However, after the removal of this 0.07 per cent sulfur, the supplemental sulfate stimulated chick growth. Therefore, the failure to observe any effect on chick growth by adding different sulfur-compounds to basal rations employed in these experiments indicates that they already contained enough inorganic sulfur.

After the completion of Experiment 15, certain feed ingredients were analyzed for total and inorganic sulfur contents. Peroxide bomb or fusion technic (Parr Instrument Co; 1950) was employed to determine total sulfur content. The inorganic sulfur content was determined by autoclaving the sample with hydrochloric acid for eight hours. It was filtered and then evaporated to a small volume on steam bath. The sulfur was determined as barium sulfate. The results are presented in Table 34.

It is noticeable that the sulfur contents of most of the material tested were rather high. Considering dehulled soybean oil meal and

TABLE 34

SULFUR CONTENT OF CERTAIN POULTRY FEED INGREDIENTS

Material	Sulfur content, %	
	Total	Inorganic
Antibiotic mixture[1]	0.44	
CFS concentrate No. 3	0.76[2] 0.98[3]	0.59[2]
Dehulled soybean oil meal	0.51	
Delamix, a trace mineral supplement	0.27	
Dried distillers solubles	0.42	
Dried whey	0.20	
Fish concentrate, Sea Horse	0.92	0.52
Fish meal, menhaden	0.93	
Ground yellow corn	0.22	
Vigofac	5.99	5.62

1 A mixture of 10, 10 and 2 pounds of TM-10, Aurofac and P-50, respectively.

2 1956 Samples.

3 1957 samples.

ground yellow corn contain 0.87 and 0.20 per cent of methionine, and 0.68 and 0.15 per cent of cystine, respectively; and assuming no other organic source of sulfur is present, it is calculated that dehulled soybean oil meal and ground yellow corn contain 0.187 and 0.043 per cent of inorganic sulfur, respectively. Consequently, only these two ingredients at the levels used in the basal rations of Experiment 5 and 13 supplied 0.13 per cent inorganic sulfur. Delamix and probably the antibiotic supplement supplied additional inorganic sulfur. This supports the conclusion that the basal rations contained enough inorganic sulfur to meet the needs of the birds.

The preceeding studies show that chicks can tolerate dietary sulfur up to 1.5 per cent without ill effect.

F. <u>Influence of urea on sulfur utilization by chicks</u>.

EXPERIMENT 16

The supplementation with inorganic sulfur showed no effect on chick growth in previous studies. Baldini and Rosenberg (1955) reported that the chick requirement for methionine increases with increased productive energy of the ration. It may be possible that the increased energy also increases the requirement for sulfur in order to meet the accelerated need for sulfur-containing amino acids.

The increased utilization of methionine hydroxy-analogue by the addition of urea was observed among chicks by Sullivan and Birds (1956). Among ruminents, the addition of inorganic sulfur increases the utilization of urea (Hunt et al., 1954; Abbert et al., 1955). Therefore, it may also be possible for supplemental urea to increase the utilization of inorganic sulfur if the chicks' requirement for sulfur-containing amino acids is raised by high energy ration.

Since the sulfur contents of certain known sources of UGF and CFS are rather high (Table 34) it was the purpose of Experiments 16 and 17 to learn if the addition of urea to ration, deficient in sulfur-containing amino acids and containing over 1000 Calories of productive energy per pound of ration, improves the utilization of supplemental sulfur.

After the completion of Experiment 8, the birds were fed a starting mash (54S-2) for one week and then used in Experiment 16. They were distributed according to weight and nutritional background. Two replicates of ten birds each were used per treatment. The experi-

TABLE 35

THE COMPOSITION OF BASAL RATIONS FOR EXPERIMENTS 16 AND 17

Ingredients	Experiment No. 16	17
	Lbs/Cwt	
Ground yellow corn	64.00	63.88
Dehulled soybean oil meal	29.11	29.20
Refined cottonseed oil	3.00	3.00
Ground limestone	1.31	1.31
Dicalcium phosphate	1.88	1.88
Iodized salt	0.50	0.50
Trace mineral mix (Delamix)*	0.10	0.10
Vitamin mix	0.10	0.13
Calculated values:		
Protein, %	20	20
Methionine, %	0.38	0.38
Cystine, %	0.29	0.29
Productive energy, Cal/lb	1005	1004
Metabolizable energy, Cal/lb	1281	1280
Vitamin mix	gm/100 lb ration	
Vitamin A (250,000 IU/gm)	1.60	1.44
Vitamin D_3 (1,500 ICU/gm)	26.00	22.70
Alpha-tocopherol acetate (20,000 ICU/lb)	4.60	4.60
Menadione	0.10	0.20
Thiamine hydrochloride	0.30	1.00
Riboflavin	0.30	0.30
Calcium pantothenate	0.50	0.50
Niacin	1.50	1.50
Pyridoxine hydrochloride	0.20	0.20
Choline chloride	10.00	25.00
Folic acid	0.10	0.25
Inositol	0.10	0.10
Vitamin B_{12} (1 mg/gm)	0.60	0.60

* See table 13.

ment continued for four weeks.

The composition of basal ration is presented in Table 35. Three levels of flowers of sulfur and urea were added, singly or in combinations. Methionine was added at 0.2 percent level as one treatment. All these materials were incorporated at the expense of ground yellow corn.

The results are presented in Table 36. Since the initial weights for different treatments were not the same they were analyzed for variance. They were not significantly different. The four-week weight gains rather than the final weights for each treatment were therefore subjected to statistical analysis. Even then, none of the treatments affected chick growth rate.

TABLE 36

THE EFFECT OF ELEMENTAL AND ORGANIC SULFUR, AND UREA ON CHICK PERFORMANCE (Experiment 16)

Treatment	Av. weight, gm initial	Av. weight, gm final	Total weight gain, gm	4-week feed efficiency
Basal	476	1266	790	2.47
0.05% sulfur	476	1249	773	2.62
0.10% sulfur	484	1262	778	2.59
0.15% sulfur	474	1257	783	2.56
0.10% urea	488	1237	749	2.62
0.20% urea	468	1256	788	2.44
0.30% urea	480	1260	780	2.50
0.05% sulfur, 0.10% urea	480	1267	787	2.50
0.10% sulfur, 0.20% urea	474	1210	727	2.64
0.15% sulfur, 0.30% urea	466	1210	744	2.50
0.2% Methionine	476	1255	779	2.41

EXPERIMENT 17

This experiment was designed with the same purpose as Experiment 16. However, slight modifications were made on the levels of supplemental vitamins in the basal ration (Table 35). Two levels of flowers of sulfur, calcium sulfate or urea were employed singly or in combinations, added at the expense of corn. Whenever calcium sulfate was used, total calcium was equalized to that of the basal ration by reducing equivalent amounts of ground limestone.

Since birds arrived four days ahead of schedule, they were fed the basal ration for the first day and distributed to the respective treatments on the following morning. Two replicates of ten birds each were used per treatment. The experiment lasted eight weeks.

The results are presented in Table 37. The final weights were analyzed for variance and found not significantly different.

These two experiments were designed on the assumption that the basal rations were deficient in sulfur-containing amino acids. It is postulated that under this condition chick requirement for inorganic sulfur may be increased by increasing the energy content of the diet and supplemental urea may improve sulfur utilization.

According to the NRC recommendation chicks require 0.45 and 0.35 per cent of methionine and cystine, respectively, on a ration containing 20 per cent protein. The basal rations employed in Experiments 16 and 17 calculated 20, 0.38 and 0.29 per cent of protein, methionine and cystine, respectively. Based on the above calculation they are deficient in these two sulfur-containing amino

TABLE 37

THE EFFECT OF SULFUR AND UREA ON CHICK GROWTH
(Experiment 17)

Source of sulfur	Urea, %	Sulfur equivalent, %	Av. final weight, gm
None	--	--	1012
Flowers of sulfur	--	0.1	1045
Flowers of sulfur	--	0.2	933
Calcium sulfate	--	0.1	1050
Calcium sulfate	--	0.2	1033
None	0.1	--	931
None	0.3	--	906
Flowers of sulfur	0.1	0.1	940
Flowers of sulfur	0.3	0.1	976
Flowers of sulfur	0.1	0.2	995
Flowers of sulfur	0.3	0.2	942
Calcium sulfate	0.1	0.1	1034
Calcium sulfate	0.3	0.1	1019
Calcium sulfate	0.1	0.2	973
Calcium sulfate	0.3	0.2	993
None*	--	--	1072

* Methionine, 0.2%

acids. However, the addition of methionine to the basal rations did not affect chick growth. Therefore, it indicates that the rations are actually not deficient in these amino acids, and the published values on nutrient contents of feedstuffs, employed for the computation, do not apply in every case. Consequently, under the experimental conditions chicks would not be expected to show benefits from the addition of flowers of sulfur, calcium sulfate or urea singly or in combinations.

G. Importance of supplemental vitamin K in rations containing corn and soybean oil meal as the only sources of protein.

EXPERIMENT 18

This experiment was originally designed to study the effect of menhaden fish meal and CFS on chick performance. An outbreak of hemorrhagic disease was encountered during the third to fourth week with 29 per cent mortality. Diagnosis of this disease was made by the Veterinary Pathology Laboratory of Michigan State University.

The composition of the basal ration, presented in Table 38, was similar to all other rations employed in this series of studies in that soybean oil meal and corn were the only protein sources. However, vitamin K was absent in the basal ration of present experiment. Therefore, it is quite possible that the lack of this vitamin was responsible for the outbreak of hemorrhagic disease. Similar rations were employed by Anderson et al. (1954) and Frost et al. (1956) to demonstrate the nutritional hemorrhagic syndrome of chicks and the curative values of different forms of vitamin K, respectively. The syndrome observed in the present experiment was very similar to that described by Anderson et al. (1954).

It should be noted that vitamin K was also not added in Experiment 2, but no hemorrhagic syndrome was observed. The discrepancy was well explained by the findings of Almquist and Stokstad (1936). Vitamin K was found to be transferred from dam to chicks through the egg yolks. Therefore, the body store of this vitamin was probably higher in the birds used in Experiment 2. Since chick growth rate is not affected directly by vitamin K deficiency until the hemorrhagic condition becomes severe (Almquist and Stokstad, 1936) the data obtained in Experiment 2 are still valid. However, the results of the present experiment must have affected and thus, cannot be used.

TABLE 38

THE COMPOSITION OF BASAL RATION FOR EXPERIMENT 18

Ingredients	Lbs/Cwt
Ground yellow corn	68.03
Dehulled soybean oil meal	28.00
Ground limestone	1.34
Dicalcium phosphate	1.92
Iodized salt	0.50
Trace mineral (Delamix)*	0.10
Vitamin mix	0.11
Vitamin mix	gm/100 lb ration
Vitamin A (10,000 IU/gm)	20.00
Vitamin D_3 (3,000 ICU/gm)	4.50
Riboflavin	0.09
Calcium pantothenate	0.20
Niacin	0.44
Choline chloride	25.70

* See Table 13

H. Other experiments

EXPERIMENT 19

The experiment was designed to compare the effects of CFS and fish meal on chick performance. The birds were reared in poultry house #500. After the completion of this house a performance test was made which indicated the existance of differences due to position of batteries in the starter-room. In the present experiment lots were randomly assigned to various batteries. At the end of this experiment, it was found that difference between batteries were highly significant. Therefore, the data for treatments were not used.

EXPERIMENT 20

This experiment was designed to study the effect of CFS and dried distillers solubles, with and without the addition of methionine and/or glycine to a high energy ration (productive energy = 1234 Cal/lb). Cage differences were found highly significant at the age of 8 weeks (growing batteries). Therefore, no interpretation is given to the results of the present experiment.

In this series of studies differences due to battery positions in the rooms of poultry house #500 were found in two out of the six experiments. Certain remedies, eg. the installation of space heaters, should be made to avoid the recurrence.

GENERAL DISCUSSION

The basal ration of Experiment 1 contained all known nutrients at, or above, the levels required by chicks for optimum growth. When CFS was incorporated in this ration, keeping the protein and metabolizable energy contents equalized, chick growth was significantly improved. This clearly indicates that CFS contains unknown growth factor(s) and belongs in that relatively small but important list of crude sources of UGF.

Like other crude UGF sources, the growth-promoting effect of CFS was modified by certain factors. It is shown in these experiments that two dietary factors influence chick response to supplemental CFS—energy level and nature of fat (Table 23). CFS stimulated growth in rations containing crude corn oil or animal yellow grease when the percentage protein, Calories of productive energy and C/P ratio, respectively, were as follows: 23:1011:44 (Experiment 1), 23:1009:44 (Experiment 7) and 22:1085:49 (Experiment 9). It did not stimulate growth when these values were: 22:935:43 (Experiment 7), 23:969:42 (Experiment 8) and 20:996:50 (Experiment 9) or when the rations contained refined cottonseed oil (Experiments 4, 5 and 6) regardless of the dietary caloric values. Therefore, the caloric value of a diet rather than its protein level or C/P ratio regulates chick response to CFS supplementation. A productive energy value of over 1000 Calories per pound of feed thus appears to be necessary for CFS to exert its best in promoting chick growth. Crude corn oil or yellow grease can be used to enhance the energy value of the ration.

The energy requirement of CFS appears to be an asset. As the energy values were increased and the C/P ratios kept about the same, the feed efficiency improved (Experiments 7 and 9). The combination of CFS and high energy rations stimulated chick growth and improved feed efficiency, respectively. This synergistic effect resulted a broiler which weighed more than three pounds at eight weeks of age on slightly more than two pounds of feed per pound of gain (Experiments 1 and 9). This efficiency is higher than that obtained by commercial broiler producers in present-day practice.

The rations used in this series of experiments were simple and easy to mix. They needed no supplemental methionine and required no further supplementation with a "fish factor". Rations of Experiment 9 were specially formulated with commercial application in mind.

Many factors are considered in formulating commercial rations— C/P ratio, sources of UGF, supplemental antibiotics, feed additives, the availability and digestibility of the nutrients, etc. The ability of CFS to promote chick growth on diets containing an antibiotic was well demonstrated in Experiments 1 and 9. The value of CFS is not limited to its UGF only. Its protein, vitamins and energy values were also utilized by the chicks (Experiments 8, 9, 11 and 12). In case the energy value of a ration falls short of 1000 Calories, the addition of CFS would raise the caloric value as well as supply UGF. The UGF content of CFS is distinct from its caloric value. For example in Experiment 1 when caloric values were equalized, CFS stimulated chick growth.

Although CFS and certain other sources of UGF contain substantial amount of inorganic sulfur (Table 34), up to 1.5 per cent of the latter in the diet did not affect chick performance. Thus, the sulfur content was not responsible for the improved growth rate of the chicks.

The wet-milling industry produces CFS year round at a rather uniform rate. This is in contrast to certain other sources of UGF which are produced seasonally. This uniform rate of production of CFS should be of considerable importance to the feed industry.

CONCLUSIONS

Twenty experiments, involving over five thousand chicks, were designed and carried out to evaluate nutritionally corn fermentation solubles (CFS Concentrate #3) and compare it with recognized sources of unidentified growth factors.

Under the experimental conditions it was found that:

CFS Concentrate #3 supplied unidentified growth factors required by chicks as well as protein, known vitamins and energy. Prolonged storage of the product reduced its growth-promoting value.

CFS Concentrate #3 replaced equivalent amounts of fish meal in the particular basal ration used.

CFS Concentrate #3 exerted its best effect in stimulating growth of meat-type chicks in diets containing over 1000 Calories of productive energy per pound of feed. It was effective in the presence of dietary antibiotic.

White Leghorn male birds were more responsive to the supplemental CFS Concentrate #3 than straight-run chicks of the same breed. A decrease in the growth rate of White Leghorn male chicks due to the addition of six per cent of refined cottonseed oil was restored by the addition of CFS Concentrate #3.

Based on the growth data, it appeared that CFS Concentrate #3 may contain the same or similar unidentified growth factor(s) as dried distillers solubles.

LITERATURE CITED

Ackerson, C. W., W. E. Ham and F. E. Mussehl, 1940. The utilization of feed elements by growing chicks. IX. The nitrogen of urea. Nab. Agr. Exp. Sta. Bul. 120:1-7.

Albert, W. W., U. S. Garrgus, R. M. Forbes and W. H. Hale, 1955. Modified urea supplements with corn silage for wintering ewe lambs. J. Ani. Sci. 14:143-152.

Almquist, H. J., and E. L. R. Stokstad, 1936. Factors influencing the incidence of dietary hemorrhagic disease in chicks. J. Nutr. 12:329-335.

Anderson, G. C., J. H. Hare, J. K. Bletner, C. E. Weakley, Jr., and J. A. Mason, 1954. A hemorrhagic condition in chicks fed simplified rations. Poultry Sci. 33:120-126.

Arscott, G. H., and G. F. Combs, 1955. Unidentified growth factors required by chicks and poults. 4. Experimental variables which influence the dietary requirements of chicks for these factors. Poultry Sci. 34:843-850.

Association of Official Agricultural Chemists, Official methods of analysis, 1955.

Baldini, J. T., and H. R. Rosenberg, 1957. The effect of calorie source in a chick diet on growth, feed utilization, and body composition. Poultry Sci. 36:432-435.

Barnett, B. D., and H. R. Bird, 1955. Standardization of assay for unidentified growth factors. Poultry Sci. 34:1178.

Barnett, B. D. and H. R. Bird, 1956. Standardization of assay for unidentified growth factors. Poultry Sci. 35:705-710.

Bice, C. M., and L. A. Dean, 1942. Utilization of urea nitrogen by growing chicks. Poultry Sci. 21:15-17.

Bosshardt, D. K., J. W. Huff and R. H. Barnes, 1956. Effect of bromine on chick growth. Proc. Soc. Expt. Biol. & Med. 92:219-221.

Brautlecht, C. A., 1953. Starch. Its sources, production and uses. Reinhold Publ. Corp. New York, N.Y.

Briggs, G. M., 1956. Inadequacy of certain salt mixtures used in studies of unidentified growth factors for chicks. Poultry Sci. 35:740-742.

Briggs, G. M., G. F. Combs, L. Friedman, J. C. Fritz, R. J. Lillie, J. W. Nelson, F. W. Quackenbush and H. W. Titus, 1956. The ANRC reference chick diet. Feed Age. May 1956, pp. 41-44.

Burns, C. H., W. W. Cravens and P. H. Phillips, 1953. The sodium and potassium requirements of the chick and their interrelationship. J. Nutr. 50:317-329.

Camp, A. A., H. T. Cartrite, J. H. Quisenberry and J. R. Couch, 1955. Further information concerning unidentified chick growth factors Poultry Sci. 34:559-566.

Camp, A. A., B. L. Reid and J. R. Couch, 1956. Growth promoting activity of ash when fed in practical diets to chicks. Poultry Sci. 35:621-626.

Camp, A. A., H. T. Cartrite, B. L. Reid, J. H. Quisenberry and J. R. Couch, 1957. Corn steepwater solubles as a source of unknown growth factor(s) for growing chicks. Poultry Sci. 36:1354-1959.

Carlson, C. W., R. F. Miller, H. T. Peeler, L. C. Norris and G. F. Heuser, 1949. The complex nature of the animal protein factor. Poultry Sci. 28:750-752.

Carlson, C. W., and E. Leitis, 1957. Methionine, betaine and choline as counteractants of selenium toxicity. Poultry Sci. 36:1108.

Combs, G. F., G. H. Arscott and H. L. Jones, 1954. Unidentified growth factors required by chicks and poults. 3. Chick studies involving practical-type rations. Poultry Sci. 33:71-79.

Combs, G. F., G. B. Sweet, H. L. Jones, G. L. Romoser and R. W. Bishop, 1954. Multiplicity of unidentified growth factors required by chicks and poults. Poultry Sci. 33:1050.

Couch, J. R., J. R. Reed, Jr., B. L. Reid and J. W. Dieckert, 1952. Distillers solubles and other sources of unidentified factors for promoting the growth of chicks and poults. Proc. Seventh Distillers Feed Conf. pp. 59-71.

Couch, J. R., J. R. Reed, Jr., R. L. Atkinson, R. W. Perrett, B. C. Welch and J. W. Dieckert, 1954. Proc. Ninth Distillers Feed Conf. pp. 38-48.

Couch J. R., B. L. Reid, A. A. Camp, W. N. Dannenburg and E. E. Rozacky, 1955. Distillers dried solubles in the diets of chicks. Proc. Tenth Distillers Feed Conf. pp. 47-57.

Dannenburg, W. N., B. L. Reid, E. E. Rozacky and J. R. Couch, 1955. An inorganic chick growth response. Poultry Sci. 34:1023-1026.

Davidson, J.A., W. N. Fischer and H. C. Zindel, 1957. Michigan broiler test No. 4, Fall 1956. Michigan Agr. Expt. Sta. Quarterly Bul. 39:594-598.

Davis, R. L., G. M. Briggs and H. J. Sloan, 1953. Effect of cobalt in diet of the chick. Proc. Soc. Expt. Biol. & Med. 82:175

Donaldson, W. E., G. F. Combs, and G. L. Romoser, 1956. Studies on energy levels in poultry rations. 1. The effect of calorie-protein ratio of the ration on growth, nutrient utilization and body composition of chicks. Poultry Sci. 35:1100-1105.

Draper, C. I., 1944. The nutritive value of corn oil meal and feather protein. Iowa Agr. Exp. Sta. Res. Bul. 326.

Duncan, D. B., 1955. Multiple range and multiple F test. Biomet. 11:1-42.

Fisher, H., H. M. Scott and R. G. Hansen, 1954. Further studies on the alfalfa factor and its relation to the liver and whey factors. J. Nutr. 52:13-24.

Fraps, G. S., 1946. Composition and productive energy of poultry feeds and rations. Texas Agr. Expt. Sta. Bul. 678.

Frost, D. V., H. S. Perdue, and H. C. Spruth, 1956. Vitamin K activity of menadione sodium bisulfite in chickens. J. Nutr. 59:181-196.

Fuller, H. L., C. W. Carrick and S. M. Hauge, 1952. A compzrison of B_{12}, fish solubles and whey in the growth of chicks. Poultry Sci. 31:473-478.

Gordon, R. S., and I. W. Sizer, 1955. Ability of sodium sulfate to stimulate growth of the chicken. Science 122:1270.

Hale, W. H., and U. S. Garrigus, 1953. Synthesis of cystine in wool from elemental sulfur and sulfate sulfur. J. Ani. Sci. 12: 492-496.

Higgins, W. S., D. A. Richert and W. W. Westerfeld, 1956. Molybdenum deficiency and tungstate unhibition studies. J. Nutr. 59:539-559.

Hill, C. H., R. L. Borchers, C. W. Ackerson and F. E. Mussehl, 1953. Studies on chick growth stimulation by alfalfa juice, casein and aureomycin. Poultry Sci. 32:775-779.

Hill, F. W., and D. L. Anderson, 1955. Comparison of productive energy and metabolizable energy determinations with chicks. Poultry Sci. 34:1201.

Hill, E. G., and G. M. Briggs, 1950. Studies on an unidentified growth promoting factor in cereals and other natural products for chicks. Poultry Sci. 29:763.

Hill, F. W., and L. M. Dansky, 1950. Studies of the protein requirements of chick and its relation to dietary energy level. Poultry Sci. 29:763.

Hill, F. W., and Ruth Renner, 1957. Metabolizable energy values of feedstuffs for poultry and their use in formulation of rations. Proc. 1957 Cornell Nutrition Conf. pp. 22-32.

Hill, F. W., D. L. Anderson and L. M. Dansky, 1954. The relation of dietary energy level to rate and efficiency of egg production. Proc., 1954 Cornell Nutrition Conf. pp. 47-52.

Hill, F. W., and L. M. Dansky, 1954. Studies of the energy requirements of chickens. 1. The effect of dietary energy level on growth and feed consumption. Poultry Sci. 33:112-119.

Hubbell, C. H., 1957. Feedstuffs analysis table. Feedstuffs.

Hunt, C. H., O. G. Bentley, T. V. Hershberger, and J. H. Cline, 1954. The effect of carbohydrates and sulfur on B-vitamins synthesis, cellulose digestion, and urea utilization by rumen microorganisms in vitro. J. Ani. Sci. 13:570-590.

Johnson, E. L., 1953. Season and carbohydrate source affect chick response to unidentified factors. Poultry Sci; 32:930-931.

Kerr, R. W., 1950. Chemistry and industry of starch. Academic press. Inc., New York, N.Y.

Kohler, G. P., and W. R. Graham, Jr., 1952. The seasonal response of chicks to an unidentified growth facgor found in fourage juice. Poultry Sci. 31:284-286.

Kurnick, A. A., B. L. Reid, R. N. Burroughs, H. D. Stelzner and J. R. Couch, 1957. Effect of distillers dried solubles and molybdenum on the growing chick. Proc. Sco. Expt. Biol. & Med. 95:353-356.

Leach, R. M., and L. C. Norris, 1957. Studies on factors affecting the response of chicks to molybdenum. Poultry Sci. 36:1136.

Leach, R. M., Jr., and L. C. Norris, 1958. Recent research on the potassium requirement of chicks and turkey poults. Proc. 1958 Cornell Nutrition Conf. pp. 54-59.

Leong, K. C., M. L. Sunde, H. R. Bird and C. A. Elvehjem, 1955. Effect of energy:protein ratio on growth rate, efficiency, feathering, and fat deposition in chickens. Poultry Sci. 34: 1026-1027.

Liggett, R. W., and H. Koffler, 1958. Corn steep liquor in microbiology. Bacteriol. Rev. 12:297-391.

Lillie, R. J., J. R. Sizemore and H. R. Bird, 1953. Unidentified factors in poultry nutrition. 1. Development of chick assay. Poultry Sci. 32:855-862.

Lillie, R. J., H. Menge, J. J. Miner and C. A. Denton, 1958. A breed difference with respect to carry-over effect of unidentified growth factors. Poultry Sci. 37:344-352.

Lowe, I. P., and K. Roberts, 1955. Incoorporation of radioactive sulfate sulfur into taurine and other substances in the chick embryo. J. Biol. Chem. 212:477-483.

Machlin, L. J., 1955. Studies on the growth response in the chick from the addition of sulfate to a low-sulfru diet. Poultry Sci. 34:1209.

Machlin, L. J., C. A. Denton, W. L. Kellogg and H. R. Bird, 1952. Effect of dietary antibiotic upon feed efficiency and protein requirement of growing chickens. Poultry Sci. 31:106-109.

Machlin, L. J.,C. A. Denton, P. B. Pearson and H. R. Bird, 1952. Synthesis of cystine from inorganic sulfate by the hen. Fed. Proc. 11:448-449.

Machlin, L. J., P. B. Pearson, C. A. Denton and H. R. Bird, 1953. The tilization of sulfate sulfur by the laying hen and its incorporation into cysine. J. Biol. Chem. 205:213-219.

Machlin, L. J., J. R. Jackson, A. H. Lankenau and P. B. Pearson, 1954. Uptake of S^{35} in the feathers, gizzard lining, liver and muscle of young chickens after injection with radioactive methionine or sulfate. Poultry Sci. 33:234-238.

Machlin, L. J., P. B. Pearson and C. A. Denton, 1955. The utilization for the synthesis of taurine in the developing chick embryo. J. Biol. Chem. 212:469-475.

March, B. E., and J. Biely, 1954. The nutritive value of fats of different origin in chick starters. Poultry Sci. 33:1069.

March, B. E., and J. Biely, 1955. Fat studies in poultry. 3. Folic acid and fat tolerance in the chick. Poultry Sci. 34:39-44.

March, B. E., J. Biely and S. P. Touchburn, 1955. Studies on an unidentified chick growth factor in dehydrated green feed. Poultry Sci. 34:968-972.

McKittrick, D. S., 1947. The interrelations of choline and methionine in growth and the action of betaine in replacing them. Arch. Biochem. 15:133-155.

Menge, H., G. F. Combs, Peng-tung Hsu and Mary S. Shorb, 1952. Unidentified growth factors required by chicks and poults. 1. Studies with chicks using purified diets. Poultry Sci. 31:237-247.

Mitchell, H. H., and T. S. Hamilton, 1929. The biochemistry of the amino acids. Chem. Cat. Co., Inc., New York, Am. chem. Soc. Monograph Series: 619.

Morrison, A. B., 1955. The present status of unidentified growth factors in poultry nutrition. Proc. 1955 Cornell Nutrition Conf. pp. 72-79.

Morrison, A. B., M. L. Scott and L. C. Norris, 1955. Evidence for an unidentified mineral required by the chick. Poultry Sci. 34: 738-740.

Morrison, A. B., R. Dan, L. C. Norris and M. L. Scott, 1956. Further evidence on the requirement of the chick for unidentified minerals. J. Nutr. 60:283-295.

Morrison, A. B., L. C. Norris and M. L. Scott, 1956. Studies on isolation of the chick growth promoting factor(s) in the ash of unidentified growth factor supplements. Poultry. 35:1160-1161.

Morrison, F. B., Feeds and Feeding, 1950. Morrison Publ. Co., Ithacal N. Y.

Moyer, A. J., and R. D. Coghill, 1946. Penicillin VIII. Production of penicillin in suface cultures. J. Bacteriol. 51:57-78.

National Research Council, 1954. Nutrient Requirements for Poultry Pub. 301.

Nesheim, M. C., and M. L. Scott, 1957. Nutritive activities of selenium for chicks and poults. Proc. 1957 Cornell Nutrition Conf. pp. 48-52.

Norris, L. C., 1954. Unidentified chick growth factors in distillers dried solubles. Proc. Ninth Distillers Feed Conf. pp. 20-28.

Norris, L. C., 1955. Recent progress on unidentified growth factors in distillers dried solubles and other feed supplements. Proc. Tenth Distillers Feed Conf. pp. 39-42.

Norris, L. C., and T. R. Zeigler, 1958. Studies on the zinc reqirements of chicks and turkep poults. Proc. 1958 Cornell Nutrition Conf. pp. 72-78.

Norris, L. C., R. M. Leach, Jr., and T. R. Zeigler, 1957. Requiements of poultry for molybdenum, zinc and other trace elements. Proc. 1957 Cornell Nutrition Conf. pp. 33-47.

Norris, L. C., R. M. Leach, Jr., and T. R. Zeigler. Recent resaerch on the mineral requirements of poultry. Proc. 13th Distillers Feed Confer. pp. 63-73.

O'Dell, B. L., M. D. Boston, J. E. Savage and A. G. Hogan, 1952. The use of isolated soybean protein in synthetic diets for chicks. Poultry Sci. 31:559-563.

O'Dell, B. L., and J. E. Savage, 1957. Potassium, zinc and distillers dried solubles as supplements to a purified diet. Poultry Sci. 36:459-460.

Parr Instrument co., Moline, Ill., Direction Booklet No. 116.

Patterson, E. L., R. Milstrey and E. L. R. Stokstad, 1957. Effect of selenium in proventing exudative diathesis in chicks. Proc. Soc. Expt. Biol. & Med. 95:617-620.

Peterson, D. W., 1950a. Some protperties of a factor in alfalfa meal causing depression of growth in chicks. J. Biol. Chem. 183:647-653.

Peterson, D. W., 1950b. Effect of sterols on the growth of chicks fed high alfalfa diets or a diet containing quillaza saponin. J. Nutr. 42:597-607.

Petersen, C. F., A. C. Wiese and A. R. Pappenhagen, 1955. Unidentified chick growth factors. 1. Purified assay diet and crude supplement response. Poultry Sci. 34:673-678.

Philips, A. G., R. H. Carr and D. C. Kennard, 1921. Green feed versus antiseptics as a preventive of intestinal disorder of growing chicks. J. Agr. Res. 20:869-873.

Radley, J. A., 1954. Starch and its derivatives. Vol. II. John Wiley & Sons Inc., New York, N.Y.

Rand, N. T., H. M. Scott and F. A. Kummerow, 1957. The utilization of fat by the growing chick. Poultry Sci. 36:1151-1152.

Rasmussen, R. A., P. W. Luthy, J. M. Van Lanen and C. S. Borruff, 1954. The dual nature of the unidentified chick growth promoting activity of distillers dried solubles. Proc. Ninth Distillers Feed Conf. pp. 29-37.

Rasmussen, R. A., P. W. Luthy, J. M. Van Lanene and C. S. Borruff, 1957. Measurement and differentiation of unidentified chick growth factors using a new semipurified ration. Poultry Sci. 36:46-54.

Reid, B. L., A. A. Kurnick, R. L. Svacha and J. R. Couch, 1956. The effect of molybdenum on chick and poult growth. Proc. Soc. Expt. Biol. & Med. 93:245-248.

Reid, B. L., R. L. Svacha, A. A. Kurnick, F. M. Salama and J. R. Couch, 1956. Unidentified growth factors and thioctic acid in chick and poult nutrition. Proc. 11th Distillers Feed Conf. 68-79.

Reid, B. L., H. D. Stelzner, R. E. Davies, R. L. Svacha and J. R. Couch, 1958. Organic unidentified growth factors in feed ingredients. Proc. 13th Distillers Feed Conf. pp. 53-62.

Roberson, R. H., and P. J. Schaible. 1958a. Zinc requirement of the chick. Science 127:875-876.

Roberson, R. H., and P. J. Schaible, 1958b. The zinc requirement of the chick. Poultry Sci. 37:1321-1323.

Rosenberg, H. R., J. T. Baldini, M. L. Sunde, H. R. Bird and T. D. Runnels, 1955. The concomitant use of fat and methionine in broiler diets. Poultry Sci. 34:1308-1913.

Savage, J. E., B. L., O'Dell, H. L. Hempster and A. G. Hogan, 1950. The requirement of the chick for an unrecognized growth factor. Poultry Sci. 29:779.

Schaible, P. J., Selma L. Bandemer and J. A. Davidson, 1938. The Manganese content of feedstuffs and its relation to poultry Nutrition. Michigan Sta. Col., Agr. Expt. Sta. Tech. Bul. 159.

Schwarz, K., J. G. Bieri, G. M. Briggs and M. L. Scott. Proc. Soc. Expt. Biol. & Med. 95:621-625.

Scott, H. M., 1955. Accessory growth factors for chicks in distillers dried solubles. Proc. Tenth Distillers Feed. Conf. pp. 43-46.

Scott, H. M., W. D. Morrison and P. Griminger, 1955. Studies on unknown growth factors in distiller dried solubles. Poultry Sci. 34:1446-1447.

Scott, M. L., 1958. Selenium as a required trace mineral in animal nutrition. Proc. 1958 Cornell Nutrition Conf. pp. 111-117.

Scott, M. L., L. C. Norris, L. S. Jensen, A. B. Morrison and P. E. Waibel, 1954. Unidentified factors required by poultry. Proc. 1954 Cornell Nutrition Conf. pp. 82-90.

Scott, M. L., J. G. Bieri, G. M. Briggsnand and K. Schsarz, 1957. Prevention of exudative diethesis by factor 3 in chicks on vitamin E-deficient torula yeast diets. Poultry Sci. 36:1155.

Slinger, S. J., W. F. Pepper, D. C. Hill and H. D. Branion, 1952. Effect of penicillin on the growth and feed efficiency of chicks fed urea. Poultry Sci. 31:1106-1108.

Snedecor, G. W., 1950. Statistical Methods. The Iowa State College Press, Ames, Iowa.

Stark, P. B., W. H. Hale, U. S. Garrigus and R. M. Forbes, 1953. The utilization of feed nitrogen by lambs as affected by elemental sulfur. J. Ani. Sci. 12:480-491.

Sullivan, T. W., and H. R. Bird, 1956. Effect of quantity and source of dietary nitrogen on utilization of methionine hydroxy-analogue by chicks. Poultry Sci. 35:1174.

Sunde, M. L., 1956. A relationship between protein level and energy level in chick rations. Poultry Sci. 35:350-354.

Tamimie, H. S., 1955. The response of chicks to an unidentified growth factor in fish products. Poultry Sci. 34:1224.

Titus, H. W., 1957. Energy values of feedstuffs for poultry. Limestone Products Corp. Am., Newton, New Jersey.

Ucko, H., 1936. Investigations into the presence and the role of bromine in the body. Biochem. J. 30:992-1000.

Vondell, R. M., and R. C. Ringrose, 1958. The effect of protein and fat levels and calorie to protein ratio upon performance of broilers. Poultry Sci. 37:147-151.

Waibel, P. E., A. B. Morrison and L. C. Norris, 1955. Production of depleted chicks by feeding maternal diets deficient in unidentified growth factors. Poultry Sci. 34:1322-1329.

Woodruff, S., and H. Klass, 1938. A study of soybean varieties with reference to their use as food. Univ. Ill. Agr. Exp. Sta. Bul. 443.