STUDIES ON FAT REQUIREMENTS OF GROWING CHICKS

by

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INTRODUCTION

Recent studies using the purified diet technique indicate that dietary sources of certain unsaturated fatty acids are necessary to maintain normal levels of organ and depot fats in the growing chick (Reiser, 1950a) and to support optimum growth (Reiser, 1950c and James, 1950).

Commercial poultry feeds are constantly becoming lower in fat content due to the increased demand for fat in other industries and improved techniques for extracting fat and fat-like substances from normal dietary ingredients. Fats high in unsaturated fatty acids are usually quite soluble in the solvents used.

Due to this high solubility of fats containing unsaturated fatty acids, the lowering fat content of commercial poultry feed ingredients and the possibility that small amounts of certain unsaturated fatty acids may be essential for the health and optimum growth of chicks, an extensive study of the role of fat in the diet of the growing chick was initiated using the purified diet technique.

A series of experiments was conducted during which attempts were made: (1) to improve the growth of chicks receiving the purified diet as compared to the K.S.C. (Kansas State College) High Efficiency broiler diet; (2) determine whether fat alone or unsaturated fatty acids are responsible for growth stimulation by hydrogenating the fats used in certain lots; (3) determine the detrimental effects of yolkectomizing day-old chicks, which removes a large portion of the body fat reserves, and to apply various experimental designs to this type of study.

Corn oil, which is a good source of the double-bond fatty acid, linoleic, was used extensively during the study. Hydrogenated corn oil, abdominal hen fat, hydrogenated abdominal hen fat, pure ethyl linoleate, saponified corn oil, glycerol and the more and less saturated portions of corn oil as separated by the method of Newey, et al.[1] were the various supplements added to the low-fat basal diet during the experiments.

REVIEW OF LITERATURE

Early workers, McCollum and Davis (1913), fed rats low-fat diets composed of casein, carbohydrates and salts. They observed a cessation of growth which could be overcome by ether-extracts of eggs and butter, but not by the addition of lard or olive oil to the diet. They concluded that it was not necessarily the fat, but substances associated with certain fats that were essential for growth in the rat. These findings may largely be attributed to the fat-soluble vitamins in view of present knowledge, but to some extent to unsaturated fatty acids. That this is true was shown by Burr and Burr (1929). After rigidly expelling fat from

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[1] Newey, et al. Industrial Engg. Chem. 42:2538. 1950.

the diet of rats, it was found that three drops daily of an unsaturated type of fat would alleviate the characteristic deficiency symptoms produced. A review of the literature reveals that to date no deficiency symptoms which can be called "characteristic" have been demonstrated for the fowl receiving a fat-free diet. Reiser (1950c) has recently described an edema characterized by subcutaneous layers of a transparent jelly-like material occurring in some chicks at four weeks of age when fed a fat-free diet.

Cruickshank (1934a) described a harder body fat produced by mutton fat, when fed to chickens, and a softer fat produced when linseed oil was incorporated into the diet. However, in a later paper, Cruickshank (1934b) demonstrated that chicken body fats are more stable in the presence of varying dietary fat than are those of the pig and the cow.

Hilditch et al. (1934) observed that hen body fats are unlike those of the quadrupeds in that they are less saturated and similar to those of marine life in that they contain some C_{20-22} fatty acids. In pointing out other interesting facts, while comparing species depot fats, they observed that chicken depot fats are qualitatively similar to those of the rodents.

It has generally been concluded that the level of fat in the diet has no marked effect upon egg production, fertility or hatchibility. Russel et al. (1941) demonstrated a tendency toward lower and less sustained egg production when a normal laying ration was extracted with ether before being fed. It was also revealed that some hens receiving a normal laying ration may go into a negative fat balance when the level of production exceeds 66.0 per cent and that healthy non-producing hens may go into a negative fat balance while receiving the extracted diet. They concluded that since the hens did not cease production abruptly, no essential nutritional factor was lacking in the diet.

Heywang (1943) devised a laying diet containing less than 1.0 per cent fat using ground rice, casein, alfalfa leaf meal, yeast, bonemeal, limestone, activated sterols and minerals. When corn oil was added to the diet to raise the fat level as high as 8.7 per cent, no change was noted in egg weight, ratio of egg weight to yolk weight or the yolk index of fresh eggs. As the level of fat was raised, production became variable and was lowered nonsignificantly. This supports the findings of Davis and Upp (1941) who found that production varied as the fat level in the diet increased above normal levels. The latter workers also found that fertility and hatchibility were not altered by the fat content of the diet.

Heywang (1942) could demonstrate no effect on hatchibility or time of occurrence of embryonic mortality when the fat content of a low-fat diet was increased from 1.0 to 8.0 per cent by the addition of corn oil.

Taylor et al. (1944) questioned whether commercial breeder mashes containing from 2.0 per cent to 2.5 per cent fat were adequate for the well-being of the breeder flock. A diet was designed which contained 8.7 per cent of fat. By extracting various proportions of the diet, graded fat levels were produced down to 1.56 per cent. The authors cited demonstrated that the fat level has no unfavorable effect upon laying hen mortality, egg production, fertility or hatchibility.

Likewise, Reiser (1950a) could not demonstrate differences in production, fertility or hatchibility due to the absence of dietary fat, using the purified diet technique. Neither were moisture, total fat, phospholipid or cholesterol content of the eggs altered by the fat-free diet. Reiser contended that the pronounced drop in the concentration of all polyunsaturated fatty acids in the egg yolk of hens on a fat-free ration is a clear indication that the hen cannot synthesize these acids from non-fat precursors. According to the author, the slow decrease during the course of the experiment of dienoic and trienoic fatty acids in the eggs indicates that they must be slowly drawn from the body fat reserves of the hen. This observation does not agree with the earlier findings of Almquist et al. (1934). When they fed malvaceous fats to laying hens, the Halphen test, which is characteristic for such fats, was positive for depot and egg fats. When yolks present at the time of ingestion of these fats were removed by normal laying or operative techniques, subsequent eggs did not give the Halphen test, although depot fats gave a strong test. Based upon these findings, these authors concluded that depot fat is not utilized to any appreciable extent in the formation of egg fat.

Early workers have generally concluded that fat is not a dietary essential for growth in chicks, but more recent research using the purified-diet technique has indicated that certain fatty acids may be essential for optimum growth of the fowl.

Russel et al. (1940) reduced the fat content of a practical growing diet to .025 per cent by extraction with diethyl ether for 150 hours. Without adding fat-soluble vitamins, it was found that chicks receiving the extracted diet weighed 769 grams at 14 weeks while chicks receiving the normal diet weighed 885 grams, a difference of 116 grams. When vitamins A and D were provided these differences became less pronounced with the low-fat lots weighing 993 grams and the lots receiving the normal diet 1,028 grams, a difference of only 35 grams. No attempt was made to correct the diet for the vitamins E and K extracted, although some chicks showed a vitamin K deficiency. They concluded that the essentiality of fat in the diet of growing chicks was questionable under the conditions of their experiment.

The following year Davis and Upp (1941) fed chicks an all-mash diet which had been extracted with isopropyl ether for 30 hours. The fat-soluble vitamins and various levels of the extracts were added back to the diet. Although growth was somewhat reduced on the fat-free diet, the differences were overcome by the time the chicks reached maturity.

Taylor et al. (1944) found that chicks from hens receiving diets with the fat content varying from 8.7 to 1.56 per cent were not affected as far as mortality was concerned during the first three weeks of rearing.

Currently, Reiser and associates, working at the Texas Station, have been studying extensively the role of dietary fat in growing chicks. Reiser and Couch (1949) using the purified diet technique found that low fat diets supplemented with Wesson oil produced growth in chicks comparable to that produced by a practical all-mash starter. Growth of those on the low-fat purified diet was inferior. Removing the residual yolk-sacs from day-old chicks produced no growth handicap despite the diet used.

Reiser (1950b) investigated the synthesis of polyunsaturated fatty acids and the interconversions of fatty acids in the growing chick. Many interesting relationships were demonstrated. When feeding the fat-free basal as such or supplemented with bayberry tallow or olein, the fat deposition of the growing chicks was the same quantitatively and qualitatively. Organ lipids were found to retain polyunsaturated fatty acids more than the carcass lipids and the phospholipids more tenaciously than the neutral fat. Diene acid was converted to tetraene and pentaene, while triene was converted to all polyethonoic acids measured. When cod liver oil was fed, the excess polyunsaturated acids were readily converted to dienoic acid. This is in keeping with Cruickshank

(1934b) who pointed out the stability of chicken depot fat in the presence of dietary highly unsaturated acids when compared to the depot fat of the cow and the pig. It is the opinion of Reiser that chicks cannot synthesize dienoic or trienoic acids, but produce higher polyunsaturated acids solely from these.

Recently Reiser has described general growth failure and high mortality in chicks which he attributed to a lack of essential fatty acids in the purified diet used. Reiser and Couch (1949) studied the effect of fat in the diet upon growth and mortality in the growing chick. Chicks grew poorly upon the fat-free basal diet, but when 4.0 per cent of Wesson oil was added growth was stimulated and comparable to lots receiving a practical starter ration. Reiser (1950c) concluded that polyunsaturated fatty acids are definitely essential nutrients for the growth of the chick. Using a highly purified fat-free basal ration or the basal plus ethyl palmitate or bayberry tallow, chicks grew poorly and generally died within four weeks. On the other hand, those fed the basal ration plus cottonseed oil or lard grew at a rate commensurate with chicks receiving a commercial type diet.

Using low-fat purified diets composed of Cerelose, Celluflour, casein, gelatin and crystalline vitamins, amino acids and minerals, James (1950) concluded that small amounts of fat stimulate growth in chicks. Kansas White Rock cockerels fed the basal diet for 21 days averaged 103.4 grams per chick, while those receiving 0.5 per cent corn oil averaged 139.8 grams, a difference which was highly significant despite the small number of five chicks per lot. One per cent, 2.0 per cent and 10.0 per cent levels of corn oil gave decreasing growth responses in the order named with only the 1.0 per cent level producing a significant response.

MATERIALS AND METHODS

All experiments were conducted in the Small Animal Research Laboratory (Bushnell Hall) on the Kansas State College campus. Room temperature was maintained, between 70 and 75 degrees Fahrenheit, by steam heat and air cooling systems. Lighting was automatically provided for 15 hours daily.

Each deck of a contact type starting battery was symmetrically divided through the center with sheet aluminum, thus making 10 pens out of a 5 deck battery suitable for small experimental lots of chicks.

Temperature, heating-unit height, feeders and waterers were adjusted in keeping with the growth and age of the chicks. For identification purposes, all chicks were wing-banded. All White Plymouth Rocks used as purebreds or in crosses were of the Kansas State College strain. Intranasal live-virus Newcastle vaccine was administered to day-old chicks in all experiments except Experiment I. All chicks were weighed at one day of age and at least weekly thereafter through the duration of the experiment. Feed consumption records were maintained.

Fats, other than corn oil, were prepared by or under the direction of Dr. R. E. Clegg of the Kansas State College Chemistry Department. Ethyl linoleate was prepared by the method of Rollet.[1] Saponified corn oil was the fat soluble material resulting from saponification of corn oil in alcoholic KOH and subsequent neutralization of the solution. The more and less saturated portions of corn oil were prepared from saponified corn oil by the method of Newey et al.[2] The unsaturated portion will be referred to as Fraction A and the more saturated portion as Fraction B. Abdominal hen fat was removed from fat hens and separated from connective tissues by slight heating.

Diets containing fat were made by adding fats to the prepared basal diet. For example, a diet containing 3.0 per cent fat was prepared by mixing 97 parts of basal with 3 parts of the fat supplement on a weight basis. The basal diets were calculated to contain vitamins and minerals in excess of amounts needed for optimum growth to allow for dilution by fat supplements. Corn oil fractions were added on the basis of yield in their preparation. Yields were 85 per cent for saponified corn oil, 66 per cent for unsaturated Fraction A and 16 per cent for saturated Fraction B. Consequently, when corn oil was added as

[1]Rollet, A. Zschr. Physiol. Chem. 62:410. 1909.

[2]Loc. cit. on p. 2.

3 per cent of the diet, these fractions were added at 2.5, 2.0 and 0.5 per cent of the diet, respectively.

Experiment I

One-hundred White Rock day-old chicks were randomized into 10 lots of equal size.

Three purified basal diets composed of ingredients low in fat were prepared. Basal I was as used by James (1950), except 115 grams of Aurofac was added in place of the 23 grams of APF (animal protein factor supplement) per 100 pounds of diet. In Basal II the salt mixture was changed and in Basal III both the salt mixture and balance of the diet were changed. The basal diets are presented in Table 1 and the salt mixtures in Table 2.

The basal diets were prepared by weighing the ingredients used in large quantities on a portable platform scales and the ingredients used in smaller quantities on a Toledo or analytical balance. The crystalline water-soluble vitamins were premixed by grinding them with Celluflour in a large mortar. The salt mixtures were prepared by grinding the individual salts in a mortar and mixing all salts thoroughly before adding them to the other ingredients of the basal. The diets were finally mixed in a small feed mixer at the College Poultry Farm for one hour and stored in tight metal containers in the Small Animal Research Laboratory. The mixer was covered to prevent dust losses. Vitab was very difficult to mix into the diet.

Table 1. Composition of the basal diets for Experiment I.

Ingredients	Basal diet I		Basal diet II		Basal diet III	
Cerelose	67.72	lb	66.13	lb	61.39	lb
Casein	18.00		18.00		22.00	
Celluflour	5.00		5.00		5.00	
Salt mixture[1]	4.00		5.59		5.59	
Gelatin	1.00		1.00		1.00	
"Vitab"[2]	1,086.00	g	1,086.00	g	1,386.10	g
"Aurofac"[3]	115.00		115.00		115.00	
Cystine	144.60		144.60		160.00	
Arginine	98.00		98.00		20.00	
Glycine	366.70		366.70		453.60	
i-inosital	45.50		45.50		45.50	
Choline Chloride	91.00		91.00		91.00	
Niacin	4.55		4.55		5.00	
Riboflavin	800.00	mg	800.00	mg	800.00	mg
Calcium Pantothenate	668.00		668.00		668.00	
Thiamine HCl	200.00		200.00		200.00	
p-amino benzoic acid	136.50		136.50		136.50	
Folic acid	72.80		72.80		72.80	
Biotin	4.54		4.54		4.54	
Pyridoxine	none		none		50.00	
Vitamin A Acetate Vitamin D_3 Vitamin K^3 (Menadione) Mixed tocopherols	-- Given orally three times per week.[4]					
Total (lbs.)	100.00		100.00		100.00	

[1]Salt mixture 1 used in Basal I; salt mixture 2 used in Basals II and III, Table 2.

[2]"Vitab"- vitamin content per cc:

Thiamine	150.0 mmg	Niacin	2,000.0 mmg
Riboflavin	10.0	Choline	8,000.0
Pantothenic	275.0	i-inosital	6,000.0
Pyridoxine	150.0	Biotin	1.2

[3]"Aurofac" guaranteed to contain 1.8 mg vitamin B_{12} and 1.8 gm of aureomycin per pound of supplement.

[4]Vitamin mixture- prepared by adding 25 gm of vitamin A acetate, 60 mg of vitamin D_3, 9 gm of mixed tocopherols and 200 mg of Menadione to 800 ml of propylene glycol.

Table 2. Composition of salt mixtures.

Ingredients	Grams per 100 pounds of diet		
	Mix 1	Mix 2	Mix 3
$NaCl$	185.6400	226.8000	226.80
KCl	212.1700	212.1700	
KH_2PO_4	548.1000	548.1000	548.10
$Ca_3(PO_4)_2$	263.4400	735.9500	735.95
$CaCO_3$	371.2900	559.2300	559.23
$MgSO_4.7H_2O$	159.1800	226.8000	159.18
Fe pyrophosphate	105.2000	6.8040	
$FeCl_2$		6.8040	
$MnSO_4.4H_2O$	.3536	10.1360	20.00
$K_2Al_2(SO_4)_4.24H_2O$	.1591	.1591	.80
$CuSO_4$	.6894	1.0000	1.00
Fe citrate			25.00
Zn acetate			.70
$CoCl_2$			.20
$NiSO_4$			.10
Total	1846.2221	2533.9531	2277.06

The fat soluble vitamins were prepared by adding the various materials listed in Table 1 to propylene glycol. Menadione went into solution with great difficulty. The solution was given orally by dropper three times weekly at the rate of one drop per dose during the first week, two drops per dose during the second week, increasing the level one drop per dose each subsequent week that the experiment progressed. The author had previously administered propylene glycol to chicks at three times the level to be used for this purpose and observed no ill effects as measured by growth and thriftiness. The experimental chicks were crossbred (New Hampshire x White Rock) and were receiving the broiler diet presented in Table 3.

To prepare purified diets for nine of the lots, three portions of each of the three basal diets were weighed out. One portion of each basal was fed as such, ethyl linoleate was added to another at a level to compose 0.25 per cent of the diet and corn oil was added to another portion of each basal at a level to compose 0.5 per cent of the diet. The tenth lot received the broiler diet presented in Table 3.

The diets containing fat were prepared twice weekly and kept under refrigeration to limit oxidation of the fat. The unused corn oil and fat-soluble vitamin concentrate were kept under refrigeration as was the ethyl linoleate. The latter was held under nitrogen before being mixed in the feed.

The experiment was terminated when the chicks were 7 weeks of age.

Table 3. Composition of K.S.C. high efficiency broiler diet.

Ingredients	Per 100 pounds
Ground yellow corn	61.5 lb
Wheat bran	4.0
Alfalfa meal (17% Dehyd.)	1.0
Soybean oil meal (44% solvent ext.)	30.0
Calcium carbonate	1.0
Steamed bone meal	2.0
Salt (NaCl)	0.5
"Prot -A"[1]	100.0 g
"Delsterol"[2]	40.0
Manganese sulfate	25.0
Riboflavin	5.0
Choline chloride (crystalline)	9.0
Calcium pantothenate	1.0
Niacin	5.0
"Aurofac"	115.0

[1] Supplies 2,400 USP units of vitamin A per gm of supplement.

[2] Supplies 2,000 A.O.A.C. units of vitamin D_3 per gm of supplement.

Experiment II

Thirty White Rock cockerels were randomized into 3 lots of 10 each.

An attempt was made to improve the purified basal diet and to reduce the fat content of the diet. Gelatin, Celluflour and casein were extracted with Skellysolve B in a large Soxhlet extractor for 24 hours. This was followed by an extraction with ethyl alcohol for a similar period. Enough material was extracted to provide ingredients for all subsequent experiments.

Gelatin was increased in the diet to reduce the amount of crystalline glycine and arginine needed and because of its growth promoting properties. Vitab was eliminated due to its tendency to form clumps with powdery portions of the diet, thus not mixing into the diet evenly. The amount of several vitamins was increased to replace the Vitab and to maintain vitamin levels at two times National Research Council recommendations. Basal IV is presented in Table 4.

Salt mixture 3, Table 2, was used in Basal IV. Ferric citrate was used as the source of iron. Salts of nickel, zinc and cobalt were added. The salts of nickel, cobalt, manganese, aluminum and zinc were dissolved in water, mixed with tricalcium phosphate and allowed to dry. The dried material was then ground in a mortar and mixed with the other minerals which had previously been ground in a mortar.

Table 4. Composition of Basal IV.

Ingredients	Per 100 pounds
Cerelose	62.56 lb
Casein	18.00
Gelatin	8.00
Celluflour	5.00
Salt mixture[1]	5.20
Cystine	180.00 g
"Aurofac"	115.00
Choline Chloride	100.00
i-inosital	45.50
Arginine	35.00
Niacin	4.55
Calcium Pantothenate	2.27
Riboflavin	1.60
Thiamine HCl	908.00 mg
p-amino benzoic acid	450.00
Pyridoxine	454.00
Folic acid	227.00
Biotin	9.08
Menadione	200.00
Fat soluble vitamin mix[2]	150.00 ml
Total	100.00 lb

[1] Salt mixture 3 as shown in Table 2.

[2] Drawn from the fat-soluble vitamin mixture prepared for Experiment I. (Table 1).

The fat-soluble vitamin concentrate was mixed directly into the basal at a level to insure excesses of all vitamins based on National Research Council Recommendations of 1950. Due to its poor solubility in both propylene glycol and water, additional Menadione was added to the diet.

The prepared basal was stored at 0° F. to reduce oxidation of the vitamins. Diets for the individual lots were prepared by feeding the basal diet alone and the basal containing 0.5 per cent and 3.0 per cent of corn oil. This portion of the experiment was terminated when the chicks were 4 weeks old.

Lots 1a and 2a were composed of 34 crossbred cockerels (New Hampshire x White Rock). Of the 17 chicks in each lot, 10 were yolkectomized as day-old chicks using the method of Sloan (1936). Lot 1a received Basal IV and Lot 2a received Basal IV containing 3.0 per cent of corn oil. This phase of the experiment was terminated when the chicks were 4 weeks of age.

Experiment III

Seventy-eight White Leghorn cockerels were randomized into six lots of 13 chicks each. Seven of the chicks from each lot were randomly chosen to be yolkectomized at one day of age.

Purified Basal IV was prepared and stored as in Experiment II. One lot received the broiler diet and another was fed Basal IV. The other four lots received Basal IV supplemented with 3.0 per cent corn oil, hydrogenated corn oil, abdominal hen fat, and hydrogenated abdominal hen fat. The experiment was terminated when the chicks were 4 weeks old.

Fecal analyses were made of the dried feces to determine the digestibility and absorption of the hydrogenated material added to the diet relative to the natural fat.

Experiment IV

Basal IV was prepared and stored as in previous experiments. One-hundred White Leghorn cockerels[1] were placed on Basal IV for two weeks to deplete the chicks of their fat reserves. Fifty-five of the chicks were yolkectomized at one day of age.

At two weeks of age, the yolkectomized group and non-yolkectomized group of chicks, were ranked from best to poorest based on individual gains during the period. Individuals with exceptionally high or low gains were eliminated until 36 individuals were left in each group, yolkectomized and non-yolkectomized. The six individuals from each group with the most uniform gains were then randomized among six lots, one yolkectomized and one non-yolkectomized chick being placed in each lot. Each group of six was considered to be identical and used for paired statistical analyses of gains at the

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[1] Generously supplied by J. O. Coombs and Son, Sedgwick, Kansas.

termination of the test period. The next six individuals in rank in each group were then randomized among the six lots. This procedure was followed until six yolkectomized and six non-yolkectomized individuals were in each lot. The uniformity of each lot assembled in this manner can be seen by studying the mean gain from 0-14 days and the standard error which is shown in Table 15 and growth graphs in Plate VI. After the 2-week depletion period the diets used consisted of Basal IV, Basal IV supplemented with unsaturated Fraction A of 3.0 per cent of corn oil, Basal IV plus saturated Fraction B of 3.0 per cent of corn oil, Basal IV containing 3.0 per cent of saponified corn oil, Basal IV plus 3.0 per cent of corn oil and Basal IV supplemented with 3.0 per cent of hydrogenated corn oil. The experiment was terminated when the chicks were five weeks of age, having received the fat supplements for three weeks.

Experiment V

Eighty-five day-old silver males (New Hampshire x White Rock) were placed on the Basal IV low-fat diet for 10 days. This allowed for depletion of the body fat reserves and for the individual chicks to establish growth curves. Basal IV was prepared and stored as in previous experiments.

At 10 days, the 84 surviving chicks were ranked from best to poorest based upon their weight gains. Those individuals which varied most from the mean 10-day gain were eliminated from the experiment until 70 chicks remained. These chicks were then divided into 7 comparable lots using the method of Experiment IV.

The seven purified diets used after the 10-day depletion period consisted of Basal IV alone and Basal IV supplemented with 1.0 and 3.0 per cent of glycerol and 0.5, 1.0, 2.0 and 3.0 per cent of corn oil.

A comparable lot of chicks received the K.S.C. High Efficiency broiler diet containing Aurofac, Table 3, throughout the experiment. This method was used to compare growth of chicks receiving a purified type diet and a practical diet. The experiment was terminated when the chicks were 28 days of age, having received the supplemented diets for 18 days.

RESULTS

All statistical analyses applied are described by Snedecor (1946).

Experiment I

<u>Growth at 49 Days</u>. The average weight of each lot, mortality and diet fed are presented in Table 5. Growth graphs are shown in Plate I and analysis of variance in Table 6.

Growth of chicks receiving the purified diets was inferior at all ages and in all cases to those receiving the broiler diet.

Table 5. Experiment I, diet, average weight and mortality at 49 days for White Rock chicks.

Lot	Diet	Weight in grams	Mortality %
1	Basal III	321.3 ± 61.1	40
2	Basal III + .25% ethyl linoleate	358.6 ± 63.5	20
3	Basal III + .50% corn oil	230.4 ± 52.4	10
4	Basal I	391.4 ± 32.9	10
5	Basal I + .25% ethyl linoleate	396.6 ± 38.4	30
6	Basal I + .50% corn oil	407.1 ± 55.6	30
7.	Basal II	471.1 ± 24.5	0
8	Basal II + .25% ethyl linoleate	550.4 ± 35.0	20
9	Basal II + .50% corn oil	427.6 ± 53.0	10
10	Broiler diet	704.6 ± 33.8	10

Table 6. Experiment I, analysis of variance of growth at 49 days of lots receiving purified diets.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Individuals	72	232,536	3,230
Basals	2	47,589	23,794**
Fats	2	3,341	1,671
Interactions	4	10,325	2,581
Total	80	293,791	

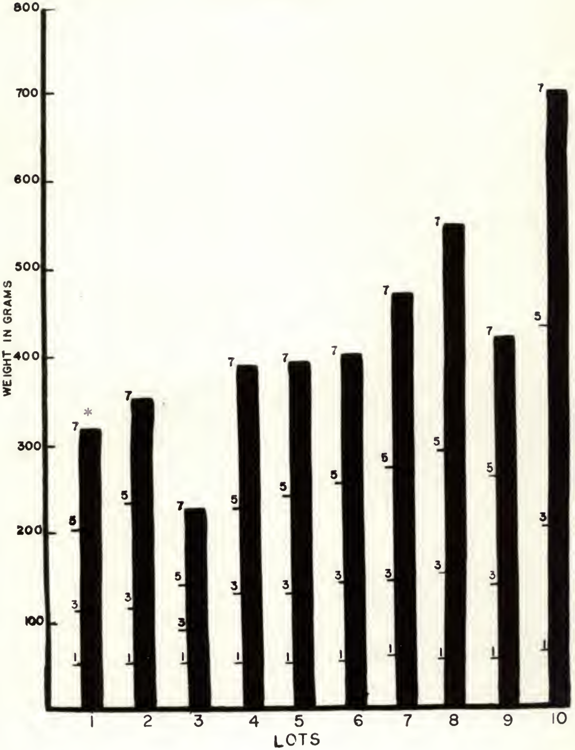
The latter chicks were also more uniform at 49 days of age as shown by the standard error of the mean lot weights.

All of the lots receiving Basal I grew better than those receiving Basal III, while the three lots receiving Basal II grew the best despite the fat supplements added to the other basals. Analysis of variance showed a highly significant difference among the basal diets used in this experiment, Table 6. Although the growth noted, using Basal II, exceeded that of Basal I, used by James (1950), it left much to be desired when compared to chicks receiving the practical broiler diet.

Responses from adding fat to the diet were variable. Ethyl linoleate gave responses in all cases. The responses were negligible in view of the large standard error except in the case of Basal II where a 79.3 gram margin was recorded. Corn oil supplementation gave negative responses in two cases and only a slight response when used with Basal I. Due to the high rate of mortality, the great amount of individual variation within lots and variable responses to the addition of fat supplements to the basal diets, no consistent growth response was noted from adding fat to the diets.

Mortality. Mortality was quite high during the experiment as indicated in Table 5. No chicks were lost in Lot 7 while four died in Lot 1. Mortality was lower in lots receiving Basal II. Many of the chicks became unthrifty and gained poorly during the final weeks of the trial. This was especially

PLATE I


800
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WEIGHT IN GRAMS
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10
LOTS

true of lots receiving Basal III, with Lot 3 containing five individuals weighing less than 200 grams each at 7 weeks of age.

Several chicks had an accumulation of mucus-like material in the crop. Upon palpation this material was normally expelled and the chick usually demonstrated no ill effects.

Necropsies revealed various abnormalities, but there were no consistent symptoms noted in any particular lot. Inflammation of the proventriculus, unabsorbed yolk sacs, enlarged streaked livers and internal hemorrhage were among the abnormalities noted.

Feed Efficiency. Feed efficiencies were calculated on a chick-day basis as grams of feed required per gram of gain. Weekly efficiencies for each lot and average figures are presented in Table 7.

Chicks receiving the broiler diet utilized feed more efficiently than those receiving purified diets. Basal III, used in Lots 1, 2 and 3, was used less efficiently than the other purified diets. Lot 8, which received Basal II plus ethyl linoleate, used feed the most efficiently. This lot also attained the greatest average weight among lots receiving the purified diets. There was no marked or consistent improvement in feed efficiency due to fat supplementation in this experiment.

Other Observations. Feathering was abnormal when chicks received purified diets. The feathers were ragged and short when compared to those of chicks receiving the broiler diet.

Table 7. Experiment I, feed efficiency for White Rock chicks (gm feed per gm gain).

Lot	Week 1	2	3	4	5	6	7	Average
1	2.87	2.73	3.21	2.96	2.91	3.27	2.84	2.97
2	2.25	2.86	3.16	2.63	2.86	3.33	3.39	2.93
3	2.41	3.41	3.99	2.93	3.91	2.70	3.24	3.23
4	2.59	2.63	2.73	3.32	2.91	2.67	2.85	2.81
5	2.64	2.63	2.54	2.77	2.82	2.67	2.82	2.70
6	2.61	2.30	2.58	2.78	2.71	2.55	3.15	2.77
7	2.16	2.80	2.53	2.81	2.82	2.67	3.14	2.70
8	2.21	2.24	2.36	3.02	2.53	2.57	2.24	2.45
9	2.35	2.76	2.45	2.69	2.78	2.62	3.53	2.74
10	2.05	2.33	2.22	2.21	2.32	2.35	2.99	2.35

Feathering was most abnormal in lots receiving Basal III. A solution of folic acid was prepared and administered three times per week during the third and fourth weeks to the lots receiving Basal III. Each dose contained 0.728 mg of folic acid. No beneficial results were noted in feather development.

Several chicks were observed which had nervous symptoms characteristic of thiamine avitaminosis. The condition was most common in lots receiving Basal III. These chicks did not respond to thiamine therapy.

Several chicks walked with a stilted gait, but did not have soft beaks characteristic of avitaminosis D. Irradiation was provided periodically with an S-4 bulb, but no recovery was noted. Apparently a multiple deficiency existed which was not corrected by the nutrients or treatment administered. Corn oil or ethyl linoleate did not protect against the development of these symptoms.

Feces were very light in color, moist, sticky and developed a strong odor if allowed to stay on the trays for more than two days. Small amounts of fecal material were eliminated as compared to chicks receiving the broiler diet.

Experiment II

Growth of Lots 1, 2 and 3. The average weight of each lot, mortality and diets fed are shown in Table 8. Growth graphs are presented in Plate II and analysis of variance in Table 9.

Table 8. Experiment II, diet, average weight and mortality at 28 days for cockerels.

Lot	Diet	Weight in grams	Mortality %
	Kansas White Rock cockerels		
1	Basal IV	318.0 ± 17.2	10
2	Basal IV + 0.5% corn oil	317.5 ± 10.8	0
3	Basal IV + 3.0% corn oil	338.1 ± 6.7	0
	Crossbred cockerels		
1a	Basal IV non-yolkectomized	321.7 ± 16.2	0
	yolkectomized	281.1 ± 15.8	0
2a	Basal IV + 3.0% corn oil non-yolkectomized	386.0 ± 24.0	0
	yolkectomized	384.0 ± 12.0	0

Table 9. Experiment II, analysis of variance of growth at 28 days for Kansas White Rock cockerels.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Individuals	26	72,173	2,776
Diets	2	2,718	1,359
Total	28	74,891	

EXPLANATION OF PLATE II

*The number beside the bar indicates the age of the chicks expressed in weeks.

Diets for lots in Experiment II

Diets for Kansas White Rock cockerels:

Lot 1. Basal IV

Lot 2. Basal IV + 0.5% corn oil

Lot 3. Basal IV + 3.0% corn oil

Diets for crossbred cockerels:

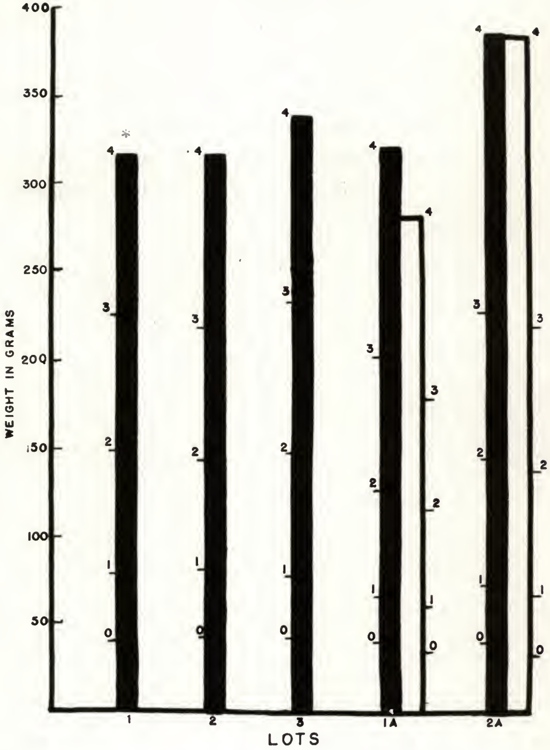
Lot 1a. Basal IV

Lot 2a. Basal IV + 3.0% corn oil

 non-yolkectomized

 yolkectomized

PLATE II



400
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WEIGHT IN GRAMS
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4
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LOTS

Growth at four weeks exceeded that of all basals used in Experiment I. Since chicks from the same breeding stock were used in both experiments, the four week weights will be compared to show the superiority of Basal IV. The male chicks in Lot 7 of Experiment I which received Basal II averaged 202.8 $\pm$ 20.56 grams at four weeks, while the cockerels receiving Basal IV in Experiment II averaged 318.0 $\pm$ 17.2 grams. When the diets contained 0.5 per cent corn oil, the figures were 247.2 $\pm$ 21.28 grams and 317.5 $\pm$ 10.8 grams, respectively. Obviously, these differences are highly significant, indicating a further improvement in the basal diet. Response to the various basal diets is shown graphically in Fig. 1.

The small response of 20 grams due to adding 3.0 per cent fat to the diet was not significant. No response was noted when 0.5 per cent fat was added to the diet. Plate II depicts the uniform and consistent growth rates for all of the lots.

<u>Mortality</u>. Only one chick died during the 28 days. This chick was only three days old at death and necropsy revealed no abnormalities. In general, the chicks appeared in a better state of health and nutrition than chicks in Experiment I. This also indicates that Basal IV was superior to all former basals used.

<u>Feed Efficiency</u>. Chicks receiving fat in their diets used feed much more efficiently. The average efficiency for the lots receiving 3.0 per cent corn oil was 1.75, while for the unsupplemented lot it was 2.15. The lot receiving 0.5 per cent corn

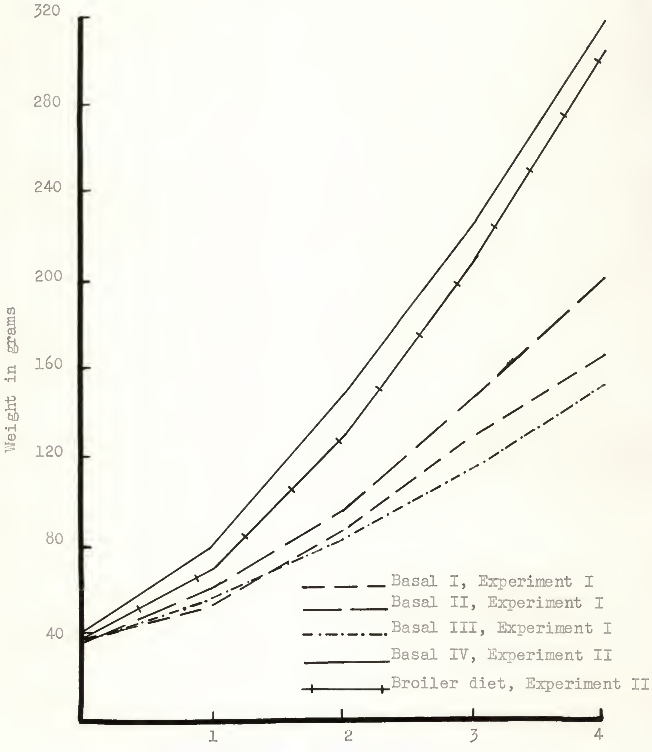


Fig. 1. Growth of White Rock cockerels.

oil had an intermediate efficiency of 1.89.

Other Observations. Feather development was normal in all lots of this experiment. No abnormal conditions were apparent during the experiment. Altering the basal diet as already described seems to have prevented the development of abnormal conditions among the experimental chicks.

Growth of Lots 1a and 2a. The average weight at 28 days, mortality and diet fed are presented in Table 8. Growth rates are shown graphically in Plate II and analysis of variance in Table 10.

Growth was excellent using Basal IV in this phase of the experiment. It was commensurate with that attained in Lots 1, 2 and 3.

Chicks receiving the diet containing 3.0 per cent corn oil gained significantly more than those with no added fat. The yolkectomized chicks receiving 3.0 per cent fat gained as well as their non-yolkectomized lot-mates. The yolkectomized chicks which did not receive fat in the diet gained at a much slower rate than their non-yolkectomized lot-mates. The difference of 40.6 grams was not significant when analyzed by the t-test. Nevertheless, this difference indicates that yolkectomized chicks may require some factor found in corn oil to gain at a rate commensurate with non-yolkectomized chicks receiving low-fat diets.

There was no mortality during this phase of the experiment. All chicks appeared vigorous and feathered normally. The yolkectomized chicks were less active for a few days following

Table 10. Experiment II, analysis of variance of growth at 28 days for crossbred cockerels.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Individuals	30	70,876	2,363
Diets	1	64,336	64,336**
Yolkectomy	1	3,738	3,738
Interactions	1	3,080	3,080
Total	33	142,021	

Table 11. Experiment II, feed efficiency for White Rock and crossbred cockerels (gm feed per gm gain).

Lot	Week				Average
	1	2	3	4	
1	1.45	1.69	2.71	2.33	2.15
2	1.52	1.75	1.95	2.07	1.89
3	1.41	1.64	1.79	1.89	1.75
1a	1.88	2.00	2.02	2.15	2.05
2a	1.51	1.68	1.77	1.77	1.73

yolkectomy, but recovered remarkably from the operation with no mortality.

Chicks receiving corn oil gained more efficiently. This difference was consistent during each week of the experiment, Table 11. The average efficiency was 2.05 for the basal diet and 1.73 for the supplemented diet.

Experiment III

Growth at 28 Days. The diet used in each lot, average weight and mortality are presented in Table 12. Growth graphs are shown in Plate III and analysis of variance of lots receiving purified diets in Table 13.

Analysis of variance shows a highly significant response in growth due to fat supplementation. These differences seem to be correlated with the unsaturation of the fat used. In all cases natural fats gave a greater growth response than their hydrogenated products. Non-yolkectomized chicks fed corn oil weighed 270.6 grams at four weeks, while those fed hydrogenated corn oil weighed 261.7 grams. The difference was more striking in the case of hen fat with the weights being 242.0 and 209.0 grams, respectively. Abdominal hen fat did not give a growth response comparable to corn oil. This is also true when hydrogenated products are compared.

The effect of yolkectomizing the chicks is partially consistent with the findings of Experiment II. Yolkectomized and

Table 12. Experiment III, diet, mortality and average weight at 28 days for White Leghorn cockerels.

Lot	Diet	Weight in grams	Mortality %
1	Basal IV		
	non-yolkecomized	195.3 ± 8.8	33
	yolkectomized	198.3 ± 9.1	0
2	Basal IV + 3% corn oil		
	non-yolkectomized	270.6 ± 16.6	16
	yolkectomized	259.6 ± 13.5	14
3	Basal IV + 3% hyd corn oil		
	non-yolkectomized	261.7 ± 16.3	0
	yolkectomized	223.2 ± 10.2	0
4	Basal IV + 3% hen fat		
	non-yolkectomized	242.0 ± 21.1	0
	yolkectomized	241.4 ± 11.0	0
5	Basal IV + 3% hyd hen fat		
	non-yolkectomized	209.0 ± 17.8	0
	yolkectomized	183.0 ± 8.9	0
6	Broiler diet		
	non-yolkectomized	188.7 ± 9.6	0
	yolkectomized	183.0 ± 1.7	0

Table 13. Experiment III, analysis of variance of growth at 28 days for White Leghorn cockerels.

Source of variation	Degree of freedom	Sum of squares	Mean square
Individuals	47	57,654	1,224
Diets	4	29,847	7,462**
Yolkectomy	1	6,952	6,952
Interactions	4	3,580	895
Total	56	98,033	

EXPLANATION OF PLATE III

The number beside the bar indicates the age of the chicks expressed in weeks.

Diets for lots in Experiment III:

Lot 1. Basal IV

Lot 2. Basal IV + 3.0% corn oil

Lot 3. Basal IV + 3.0% hydrogenated corn oil

Lot 4. Basal IV + 3.0% abdominal hen fat

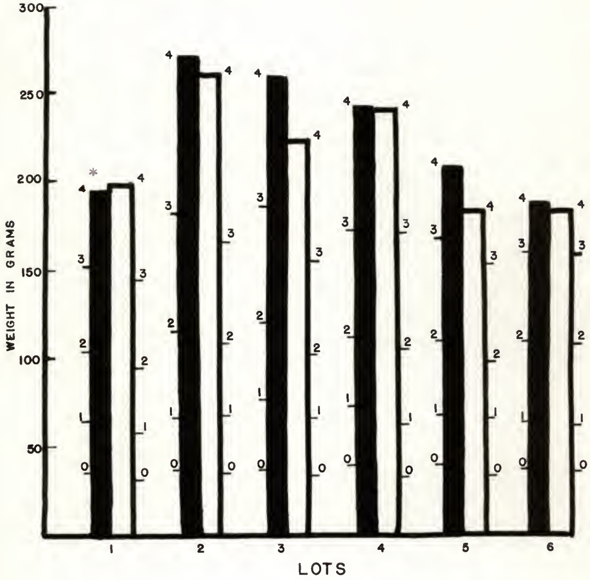
Lot 5. Basal IV + 3.0% hydrogenated abdominal hen fat

Lot 6. K.S.C. High Efficiency broiler diet.

■ non-yolkectomized

□ yolkectomized

PLATE III



non-yolkectomized lot-mates grew at comparable rates when natural corn oil or hen fat were added to the diet. When hydrogenated materials were added, performance of the yolkectomized chicks was inferior compared to their lot-mates. When corn oil was added to the diet, non-yolkectomized chicks averaged 270.6 grams and yolkectomized chicks 259.6 grams at 28 days, a difference of only 11.0 grams. When hydrogenated corn oil was fed, the weights were 261.7 and 223.2 grams, a difference of 38.5 grams. Yolkectomized chicks receiving hen fat weighed 242.0 grams and their lot-mates 241.4 grams, while those receiving the hydrogenated material weighed 209.0 and 183.0 grams, respectively, a difference of 26 grams. Growth of chicks receiving the basal diet, Lot 1, was inferior, but the yolkectomized chicks grew at a rate commensurate with non-yolkectomized chicks.

Growth of chicks fed the broiler diet was inferior to all lots receiving the purified diets. The poor growth of these chicks cannot be explained. Yolkectomized chicks demonstrated no growth handicap when fed the broiler diet.

Mortality. Mortality was quite low during the experiment. Individuals dying within 72 hours following yolkectomy were considered to have succumbed to operative shock and were not charged against mortality. Five individuals were lost in this manner. No evidence of disease was noted and necropsies revealed no abnormalities.

Feed Efficiency. Chicks fed the purified diets utilized feed more efficiently than chicks fed the broiler diet. Chicks receiving only the basal used feed less efficiently than those fed fat supplements. The efficiency was best when natural fats were used. The hydrogenated supplements gave efficiencies intermediate in nature, Table 14.

Other Observations. On the final day of the experiment, duplicate samples of feces were drawn from dropping pans of lots receiving the basal diet, basal diet containing hydrogenated corn oil and the lot receiving the corn oil supplement. The feces were dried for 12 hours in a drying oven, weighed onto cotton, placed in an extraction thimble and extracted for 12 hours in a Soxhlet extractor with anhydrous diethyl ether. The boiling flask was freed of solvent in vacuo and extracted material determined by change in the weight of the boiling flask.

After averaging the two determinations, it was found that feces from chicks receiving hydrogenated corn oil contained 15.8 per cent of ether extractables on a dry matter basis. The lots receiving the basal alone and the basal plus corn oil had a content of 1.72 and 2.80 per cent, respectively. Evidently the saturated material is not as readily available to the chick as the natural product.

Several chicks were photographed at 28 days of age. Plate IV shows the appearance of chicks receiving the purified diet as compared to chicks receiving the broiler diet. A yolkectomized chick is shown in Fig. D to demonstrate the appearance and

Table 14. Experiment III, feed efficiency for White Leghorn cockerels (gm feed per gm gain).

Lot	Week 1	2	3	4	Average
1	2.05	2.10	2.22	2.81	2.35
2	1.71	1.78	1.98	2.27	1.99
3	1.77	2.07	2.29	2.46	2.22
4	1.93	1.92	2.06	2.26	2.08
5	1.81	2.06	2.42	2.68	2.32
6	2.05	2.02	2.83	3.40	2.60

EXPLANATION OF PLATE IV

White Leghorn cockerels from various lots in Experiment III. Chicks pictured are those nearest the lot mean weight at 28 days.

Fig. 1 This cockerel received the K.S.C. High Efficiency broiler diet and weighed 183 grams. Note that appearance is comparable to that of chicks receiving the purified diets.

Fig. 2 This cockerel received Basal IV and weighed 203 grams. The chick does not show any marked nutritional failure.

Fig. 3 This non-yolkectomized cockerel received Basal IV + 3.0% corn oil and weighed 275 grams. The cockerel is heavier than the one which received Basal IV.

Fig. 4 This yolkectomized cockerel received Basal IV + 3.0% corn oil and weighed 242 grams. Note the remarkable recovery from yolkectomy and appearance which is similar to that of other chicks pictured.

PLATE IV

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

recovery of yolkectomized chicks. Plate V demonstrates the normal appearance of chicks receiving natural and hydrogenated fats. It is apparent that no marked nutritional failure occurs when limited amounts of unsaturated fatty acids are fed to the chick.

Experiment IV

Weight Gains at 35 Days. All chicks were reared as one lot and received the low-fat basal diet for depletion purposes during the first two weeks. When two weeks of age the chicks were paired and divided into six lots based upon their growth curves, so that all lots had approximately the same average gain. The gain of each lot from zero to two weeks and from two to five weeks and the diet fed from two to five weeks are presented in Table 15. Gains at two weeks are shown to demonstrate uniformity among lots as made up at that time.

Graphs are found in Plate VI and analysis of variance in Table 16. The supplements which contained greater amounts of unsaturated fat gave a marked growth response in all cases. During the 21-day period of supplementation the lots receiving corn oil, saponified corn oil and unsaturated Fraction A gained 247.4, 266.3 and 240.5 grams, respectively. Those receiving Fraction B, hydrogenated corn oil and no fat supplement gained 209.9, 203.9 and 192.6 grams, respectively.

EXPLANATION OF PLATE V

White Leghorn cockerels from various lots in Experiment III. Chicks pictured are those nearest the lot mean weight at 28 days.

Fig. 1 This cockerel received Basal IV + 3.0% corn oil and weighed 275 grams.

Fig. 2 This cockerel was fed Basal IV + 3.0% hydrogenated corn oil and weighed 270 grams. Growth approached that of the chick in Fig. 1 which received the natural corn oil.

Fig. 3 This cockerel was fed Basal IV + 3.0% abdominal hen fat and weighed 241 grams. Growth was slightly inferior to that received using the same level of corn oil supplementation.

Fig. 4 This cockerel received Basal IV + 3.0% hydrogenated abdominal hen fat and weighed 219 grams. Growth was inferior, but the chick appears alert and in good health.

PLATE V

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Table 15. Experiment IV, diet and average gain for White Leghorn cockerels.

Lot	Diet	Gain in grams: 0 to 14 days	14 to 35 days
1	Basal IV + corn oil		
	non-yolkectomized	77.8 ± 3.5	261.0 ± 11.3
	yolkectomized	70.2 ± 5.2	233.8 ± 20.0
2	Basal IV + Sap. corn oil		
	non-yolkectomized	76.0 ± 3.9	262.2 ± 21.3
	yolkectomized	68.8 ± 3.3	270.4 ± 9.1
3	Basal IV + Fraction A		
	non-yolkectomized	76.8 ± 3.1	253.7 ± 27.1
	yolkectomized	70.2 ± 3.9	227.2 ± 22.1
4	Basal IV + Fraction B		
	non-yolkectomized	78.8 ± 4.1	210.5 ± 26.8
	yolkectomized	69.3 ± 4.4	209.2 ± 34.1
5	Basal IV + hyd corn oil		
	non-yolkectomized	76.8 ± 3.9	224.2 ± 15.2
	yolkectomized	68.6 ± 3.3	183.5 ± 19.8
6	Basal IV		
	non-yolkectomized	77.7 ± 3.5	194.5 ± 13.0
	yolkectomized	70.5 ± 4.5	190.7 ± 22.1

EXPLANATION OF PLATE VI

*The number beside the bar indicates the age of the chicks expressed in weeks.

Diets for lots in Experiment IV:

Lot 1. Basal IV + 3.0% corn oil

Lot 2. Basal IV + saponifiables of 3.0% corn oil

Lot 3. Basal IV + unsaturated Fraction A of 3.0% corn oil

Lot 4. Basal IV + saturated Fraction B of 3.0% corn oil.

Lot 5. Basal IV + 3.0% hydrogenated corn oil

Lot 6. Basal IV

non-yolkectomized

yolkectomized

PLATE IV

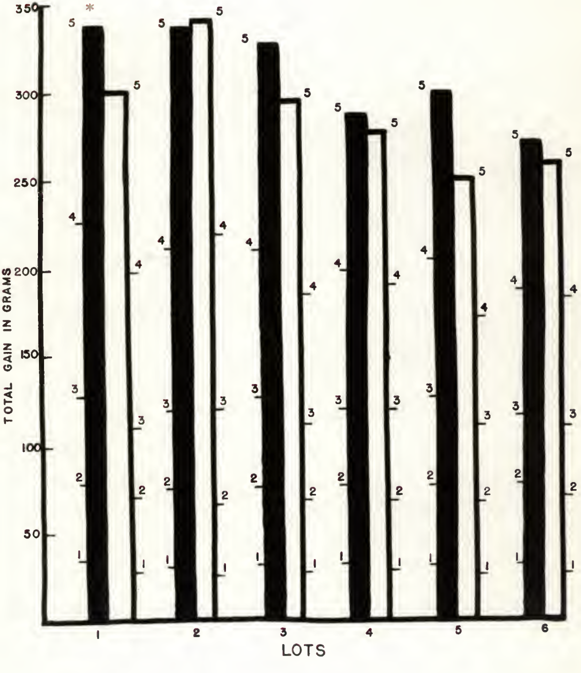


Table 16. Experiment IV, analysis of variance of gains from 14 to 35 days for White Leghorn cockerels.

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Individuals	60	163,703	2,728
Diets	5	53,788	10,758**
Yolkectomy	1	3,472	3,472
Interactions	5	6,644	1,329
Total	71	227,607	

When lot gains were broken down to those of yolkectomized and non-yolkectomized groups and analyzed by the t-test for paired individual gains, corn oil, saponified corn oil and Fraction A supplements gave highly significant responses in all cases, Table 17. The gains of yolkectomized chicks when compared to their non-yolkectomized lot-mates, are comparable in lots receiving saponified corn oil, Fraction B and the unsupplemented basal diet. Although the gain of yolkectomized chicks in the other lots was inferior, it was not significantly so.

Mortality. Five of the yolkectomized chicks died during the depletion period. All of the deaths occurred within 72 hours following the operation and were assumed to have resulted from the operation. No chicks died during the 21 days when fat supplements were fed.

Feed Efficiency. As in the two previous experiments, feed efficiency was improved when unsaturated fats were added to the diet. Average efficiencies for the 21 day test period ranged from 2.10 for Lot 1, which received corn oil, to 2.70 for Lot 6 which received the basal diet. Lots 2 and 3 which received saponified corn oil and Fraction A gave efficiencies superior to lots receiving Fraction B and hydrogenated corn oil. Without exception, the lots which showed the greatest gains also gained the most efficiently, Table 18.

Table 17. Experiment IV, t-test for paired individual gains of White Leghorn cockerels from 14-35 days.

Diets compared to Basal IV	Value of t
Non-yolkectomized	
Basal IV plus corn oil	27.82**
Basal IV plus sap. corn oil	24.98**
Basal IV plus Fraction A	23.63**
Yolkectomized	
Basal IV plus corn oil	17.14**
Basal IV plus sap. corn oil	28.36**
Basal IV plus Fraction A	14.70**

Table 18. Experiment IV, feed efficiency for White Leghorn cockerels (gm feed per gm gain).

Lot	Week			Average
	3	4	5	
1	2.40	1.96	2.09	2.10
2	2.46	1.98	2.13	2.14
3	2.29	2.36	2.13	2.24
4	2.42	2.10	2.50	2.34
5	2.50	2.44	2.49	2.47
6	2.72	2.57	2.80	2.70

Experiment V

<u>Weight Gains at 28 Days</u>. All chicks were fed the basal diet for 10 days to deplete fat reserves and to allow individuals to establish rate of gain. The chicks were then divided into seven uniform lots of paired individuals as described in Experiment IV. Gains during the depletion and supplemental periods are compiled in Table 19. Graphs are presented in Plate VII.

Glycerol was added to the basal diet to reduce dustiness and consequently make the diet more palatable without adding fat to the diet. Corn oil and other unsaturated supplements used had reduced the dustiness. It was felt that part of the growth responses noted in previous experiments may have been due to increased palatability of the diet.

Glycerol had a depressing effect upon the rate of gain. The effect was cumulative as 3.0 per cent glycerol depressed gains more than the 1.0 per cent level, Table 19.

Analysis of variance indicates highly significant differences among the diets fed, Table 20. This is partially due to the depression of gain resulting from feeding glycerol and to gain responses from fat supplementation in some lots. When 0.5 per cent or more of corn oil was incorporated in the diet, gains increased considerably. When paired individual gains were analyzed by the t-test, all of the lots receiving fat showed significantly greater gains when compared to gains of chicks fed the basal diet, Table 21. When 0.5 per cent corn oil was

Table 19. Experiment V, diet and average gain for crossbred chicks.

Lot	Diet	Average gain in grams 0-10 days	10-28 days
1	Basal IV	75.2 ± 2.46	216.6 ± 5.83
2	Basal IV + 1.0% glycerol	75.4 ± 2.69	202.2 ± 9.76
3	Basal IV + 3.0% glycerol	75.2 ± 2.48	195.6 ±12.77
4	Basal IV + 0.5% corn oil	75.3 ± 2.55	228.5 ± 9.08
5	Basal IV + 1.0% corn oil	74.9 ± 2.44	261.1 ±15.37
6	Basal IV + 2.0% corn oil	75.3 ± 2.64	233.8 ± 8.67
7	Basal IV + 3.0% corn oil	75.1 ± 2.43	233.6 ±14.60

Table 20. Experiment V, analysis of variance of gains from 10 to 28 days for crossbred cockerels.

Sources of variation	Degrees of freedom	Sum of squares	Mean square
Individuals	63	81,090	1,287
Diets	6	29,255	4,876**
Total	69	110,345	

Table 21. Experiment V, t-test for paired individual gains of crossbred chicks from 10-28 days.

Diets compared to Basal IV	Value of t
Basal IV plus 0.5% corn oil	7.30**
Basal IV plus 1.0% corn oil	20.23**
Basal IV plus 2.0% corn oil	9.50**
Basal IV plus 3.0% corn oil	7.36**

EXPLANATION OF PLATE VII

*The number beside the bar indicates the age of chicks expressed in days.

Diets for lots in Experiment V:

Lot 1. Basal IV

Lot 2. Basal IV + 1.0% glycerol

Lot 3. Basal IV + 3.0% glycerol

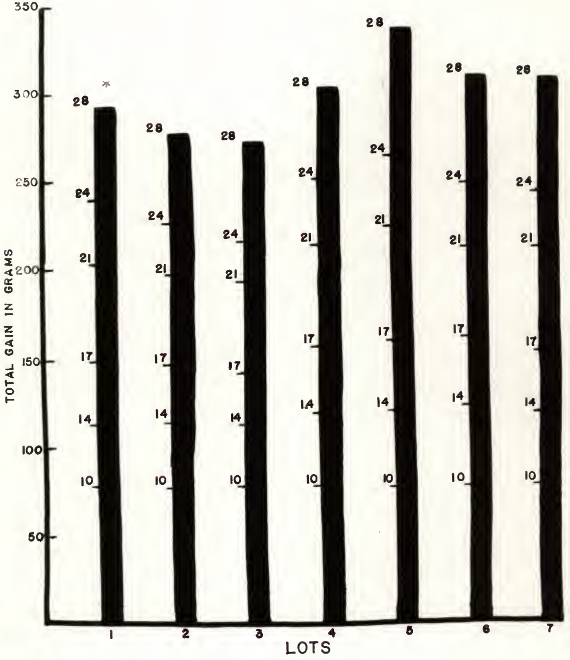
Lot 4. Basal IV + 0.5% corn oil

Lot 5. Basal IV + 1.0% corn oil

Lot 6. Basal IV + 2.0% corn oil

Lot 7. Basal IV + 3.0% corn oil

PLATE VII


350
300
250
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TOTAL GAIN IN GRAMS
*
28
24
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LOTS

fed, 8 of the 10 chicks gained more than their mates which received the basal diet during the supplemental period. When 1.0, 2.0 and 3.0 per cent fat was added 9, 7, and 7 of the 10, respectively, gained better than their mates fed the basal diet.

The levels of corn oil fed produced consistent increases in gain. One per cent corn oil gave the greatest response and appears to be the most desirable level to use with the low-fat basal diet. Lots receiving 2.0 and 3.0 per cent corn oil gained at a rate commensurate with those receiving 0.5 per cent corn oil.

Mortality. Only one chick died during the depletion period. No chicks died during the supplemental period. Feathering appeared normal in all lots.

Feed Efficiency. Feed efficiency was improved when fats were added to the diet, Table 22. Efficiency was less favorable when glycerol was added to the diet. Average efficiency during the supplemental period for the lot receiving Basal IV was 2.11. Efficiency for those receiving 0.5 per cent corn oil was also 2.11, but the values became more favorable when more fat was added to the diet. Values of 2.46 and 2.25 for the lots fed 1.0 and 3.0 per cent glycerol are definitely inferior to those recorded for the basal diet.

Other Observations. Necropsies often revealed underdeveloped digestive tracts in all experiments.

At the close of the experiment most chicks were placed on a broiler diet, but one lot was maintained on the purified diet for

Table 22. Experiment V, feed efficiency for crossbred chicks (gm feed per gm gain).

Lot	Days			
	10-17	17-25	25-28	10-28
1	1.75	2.18	2.51	2.11
2	2.09	2.51	2.43	2.46
3	1.89	2.34	2.52	2.25
4	1.75	2.18	2.51	2.11
5	1.74	2.11	2.02	1.96
6	1.62	2.33	2.17	2.03
7	1.83	2.18	2.02	2.02

an additional week. At five weeks of age, three chicks of comparable weights were chosen for alimentary tract observations. One cockerel had received the purified diet continuously, Plate VIII, another received the purified diet for four weeks and the broiler diet for one week, Plate IX, and the third chick was fed the broiler diet continuously, Plate X.

Four representative chicks were chosen from each of six lots for body analyses. Individuals chosen were those nearest the lot mean weight based on 28 day weights and were chosen from lots receiving Basal IV, Basal IV plus the four levels of corn oil and from a comparable lot of chicks receiving the broiler diet. The chicks were starved four hours and sacrificed by breaking the neck and allowing the bird to bleed into the neck region. The feathers were plucked and down removed by singeing the bird. Tarsometatarsus were removed at the hock and the resulting speciman processed through a food chopper. The body thus prepared was blended in a Kenmore blender for two minutes. Samples were taken to the analysis laboratory for moisture, nitrogen and ether extract determinations, A.O.A.C. methods being used for the analyses.[1]

The results of the four determinations for each diet were averaged and the standard errors of the means determined, Table 23. The per cent moisture did not vary significantly among the

[1] Assoc. Official Agr. Chem., Methods of analysis, 6th ed. Menasha, Wisc. Banta. 1945.

EXPLANATION OF PLATE VIII

The comparative development of the digestive tracts of chicks receiving purified and normal broiler diets. Plates VIII, IX and X were taken from the same distance and of chicks of comparable body weights.

This plate is the digestive tract of a crossbred cockerel fed purified Basal IV + 3.0% corn oil in Experiment V. The chick weighed 403 grams at five weeks when sacrificed. Note the general underdevelopment of the tract when compared to Plates IX and X. The ceca and gizzard show the greatest underdevelopment.

PLATE VIII

EXPLANATION OF PLATE IX

The comparative development of the digestive tracts of chicks receiving purified and normal broiler diets. Plates VIII, IX and X were taken from the same distance and of chicks of comparable body weights.

This plate is the digestive tract of a crossbred cockerel fed purified Basal IV + 2.0% corn oil four weeks. The broiler diet was fed from the 4th to 5th week. The chick weighed 408 grams when sacrificed at five weeks of age. Note the marked development of the gizzard, small intestine and ceca which occurred within one week. Compare to Plate VIII to note this development.

PLATE IX

EXPLANATION OF PLATE X

The comparative development of the digestive tracts of chicks receiving purified and normal broiler diets. Plates VIII, IX and X were taken from the same distance and of chicks of comparable body weights.

This plate is the digestive tract of a crossbred cockerel fed the broiler diet for five weeks. The chick weighed 401 grams when sacrificed. Note the larger more rugged appearing sections of the digestive tract when compared to chicks receiving purified diets or purified diets to four weeks of age followed by one week receiving the broiler diet, Plates VIII and IX.

PLATE X

Table 23. Experiment V, body analysis of crossbred chicks.

Diet	Per cent Moisture	Per cent Ether extract	Per cent Nitrogen
Basal IV plus			
Nothing	72.30 ± .46	6.79 ± .43	2.78 ± .14
0.5% corn oil	69.71 ± 1.29	9.53 ± 1.42	2.83 ± .05
1.0% corn oil	71.13 ± .90	9.30 ± .85	2.85 ± .04
2.0% corn oil	71.36 ± 1.06	9.63 ± 1.01	2.70 ± .06
3.0% corn oil	70.41 ± 1.02	9.83 ± 1.28	2.84 ± .12
Broiler diet	71.94 ± 1.26	8.61 ± .74	2.77 ± .02

lots as can be seen by studying the standard error of the means. Chicks receiving only the basal diet contained slightly more water and slightly less ether extractables than those receiving the diets containing fats. Since the standard error of the means are small in these two instances, these data indicate that small differences may exist. The diet containing 0.5 per cent fat results in fat deposition comparable to higher levels of dietary fat. The per cent of nitrogen in the chicks did not vary appreciably among the lots and no relationship between nitrogen deposition and fat supplementation can be noted.

DISCUSSION

Experiment I was designed primarily to improve the low-fat basal diet in terms of growth and feather development produced. The changes made in this attempt can be seen by studying Table 1. Altering the mineral mixture improved the basal diet for growth. This was achieved by raising the level of NaCl to 0.5 per cent of the diet and bringing calcium and phosphorous into a more desirable balance. Ferrous chloride, a more soluble form of iron, replaced most of the ferric pyrophosphate. Manganese was greatly increased to meet National Research Council recommended allowances.

Ethyl linoleate was added at one-half the level of corn oil used, as corn oil is about 50 per cent linoleate. If growth had been comparable and consistent using ethyl linoleate to that achieved using twice the level of corn oil, linoleic acid could be considered the growth promoting agent in corn oil. Growth was too variable within lots and responses varied too greatly with the particular basal diet used to prove or disprove this possibility.

The results do not affirm the findings of James (1950), as 0.5 per cent of corn oil did not give a marked growth response when added to Basal I.

When the casein level was increased to 22 per cent, a reduction of growth was noted. The other small changes made in the diet may have contributed to the marked growth suppression noted using Basal III.

Experiment II was designed to improve the basal diet. This was achieved, as shown in Fig. 1, by replacing much of the crystalline amino acids with natural gelatin. Vitab was replaced by crystalline vitamins and the mineral mixture changed as already indicated.

Corn oil did not produce marked growth responses, but did make the chicks grow at a more uniform rate as measured by standard error of the mean.

Reiser and Couch (1949) contended yolkectomized chicks showed no handicap in growth. When yolkectomized crossbred chicks were fed Basal IV in Experiment II, their growth was considerable below that of their non-yolkectomized lot-mates, but not significantly so. In Experiments III and IV, yolkectomized chicks fed Basal IV grew at a rate commensurate with their lotmates. These findings confirm the work of Reiser and Couch.

Crossbred cockerels responded to fat supplementation significantly in Lot 2a of Experiment II. These findings are surprising in that White Rock cockerels in Lot 3 receiving feed from the same Basal IV preparation did not respond significantly when comparable levels of fat were added.

In Experiment III hydrogenated products were used to determine whether fat alone or unsaturated fatty acids produce growth response. The results indicate that growth responses are comparable in yolkectomized and non-yolkectomized chicks when natural fats are added to the diet. When hydrogenated products are used, the non-yolkectomized chicks give a greater response than the yolkectomized chicks. This indicates that hydrogenation destroys some factor or factors in natural fats needed for yolkectomized chicks to grow at a rate commensurate with non-yolkectomized chicks. The above is true for corn oil and abdominal hen fat and the results are affirmed for corn oil in Experiment IV. In no instance are these differences significant, but they are consistent.

Fecal analyses indicate that hydrogenated materials are not readily available to the chick as measured by the per cent of ether extractables in the feces. The tests run were few and only indicative. A complete digestion trial is needed to accurately determine the digestability of hydrogenated materials as compared to nonhydrogenated materials by the chick.

Experiment IV demonstrates the additive effect of the fractions of corn oil in promoting gain increments above that achieved using the basal diet alone. When the response from unsaturated Fraction A is added to the response from saturated Fraction B, the total approximates that of corn oil. Fraction A gives the bulk of the response but was fed at four times the level of Fraction B based upon its yield in preparation. A growth response of 16 grams was noted for Fraction B, while a response of 59.2 grams was obtained from Fraction A. This indicates that the growth stimulating factors may be found equally in Fractions A and B and that the effect is additive. When paired gains are considered during the supplemental period, in 5 out of 6 pairs, the one receiving Fraction A exceeded its mate receiving Fraction B in gain. The above statements pertain to yolkectomized chicks only.

Saponified corn oil gave responses equal to and in the case of yolkectomized chicks in excess of that noted for corn oil. During the process of its preparation, most of the glycerol is lost from the corn oil. In Experiment V, glycerol proved to be a growth depressant. The significance of these findings is not known.

Experiment V was designed to determine the level of fat needed for optimum gains using the depletion and paired individual technique described. The findings affirm those of James (1950) as 0.5 per cent corn oil gave significant gain responses when compared to the basal diet gains.

The peculiar development of the digestive tract of chicks receiving a purified diet is interesting and should be of concern to nutritionists using the purified diet technique. The retarded development may interfer with the ability of the chick to digest and absorb certain supplements or compounds being tested. Thus, findings using purified diets cannot justifiably be said to indicate the true situation using a natural diet.

Feed efficiencies were consistently improved when fats were added to Basal IV. High rates of mortality in Experiment I and several unthrifty chicks made efficiency difficult and arbitrary in calculation. The greater efficiency might be explained on the basis of the chick using preformed dietary fat to lay down depot fats more efficiently than it can form fats from carbohydrates found in the purified diets. Also, as suggested by various workers cited, feeding efficiency may be improved by providing fatty acids essential for normal efficient metabolism of the growing chick.

It was decided that body analyses would aid in determining which of the above possibilities is the most likely. Analyses revealed similar body composition when fat was added to the diet at levels from 0.5 to 3.0 per cent. The slight increase in body moisture of chicks receiving only the basal diet agrees with the findings of Reiser (1950c) who found edema in such chicks. All of the gain response noted cannot be attributed to fat deposition indicating that other tissue growth was also stimulated when fat was added to the diet. Evidently, the 0.5 per cent level of supplementation provided all fatty acids in ample quantities to stimulate maximum tissue and fat deposition. Excess fats provided by feeding higher levels were evidently utilized for energy and not stored as such. This may account for increased feed efficiencies as the level of supplementation increases.

No marked growth failure or mortality occurred as noted by Reiser (1950c), when the low-fat diet was used. However, Reiser used chicks from hens fed fat-free diets for 15 weeks prior to incubation of the eggs. Growth of chicks receiving the supplemented Basal IV diet exceeded that attained by Reiser using a supplemented diet.

SUMMARY

A series of five experiments was conducted to study various aspects of the necessity of dietary fats for growing chicks.

Experiment I was an attempt to improve a purified diet for fat studies and to determine whether ethyl linoleate is the growth stimulating fraction of corn oil. Altering the salt mixture improved the basal diet found in the literature in terms of growth, mortality and feather development. Growth was still

inferior to that of chicks fed practical broiler rations. Results of fat supplementation varied with the basal used.

Experiment II was designed to further improve the basal diet, determine the response from 0.5 per cent and 3.0 per cent fat levels and to determine the effects of yolkectomizing day-old chicks on fat requirements. White Rock cockerels did not respond significantly to fat supplementation, but crossbred chicks responded, especially the yolkectomized individuals. Growth was excellent and approached that expected of chicks fed a practical broiler diet.

In Experiment III, natural fats were hydrogenated and yolkectomized and non-yolkectomized chicks were used. When fed natural supplements, non-yolkectomized and yolkectomized chicks grew at commensurate rates, but when fed hydrogenated products the growth response of yolkectomized chicks was inferior. All fat supplements produced a growth response and growth on the basal diet exceeded that of the broiler diet.

Experiment IV was designed to determine what fraction of corn oil contains the growth promoting agents. Response from the less and more saturated portions of corn oil indicate the responses are additive and not concentrated in either portion. The depletion period and supplemental period technique of paired individual gains was initiated and proved of value in small experimental lots of chicks.

Experiment V results show glycerol to be a growth depressant and that 0.5 per cent levels of corn oil make for gains comparable to 3.0 per cent corn oil. Corn oil supplementation at 0.5 per cent of the diet slightly reduced the moisture content and increased the fat content of the chick as much as higher levels of fat. Marked underdevelopment of the digestive tract resulted when purified diets were fed.

Observations and conclusions resulting from these experiments are:

1. Basal IV produced growth and feather development comparable to that attained from proved broiler diets.

2. Natural fats produce maximum growth responses in chicks. Hydrogenated products give a smaller response, especially in yolkectomized chicks. Hydrogenated materials are less available to the chick.

3. Using Basal IV and the depletion and paired-gain technique, 0.5 per cent corn oil gives growth responses comparable to 3.0 per cent corn oil.

4. Growth promoting substances may be found in equal concentration in unsaturated and more saturated portions of corn oil when fractionated by method of Newey et al.[1]

5. Feed efficiencies are consistently more favorable when fat is added to otherwise adequate purified low-fat diets.

6. Marked underdevelopment of the digestive tract resulted from feeding purified diets.

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[1] Loc. cit. on p. 2.

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STUDIES ON FAT REQUIREMENTS OF GROWING CHICKS

by

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A series of 5 experiments was conducted to study various aspects of essential fats for growing chicks using the purified diet technique. Chicks were battery reared and normal husbandry practices were followed. Basal low-fat diets found in the literature were altered until growth and feather development using Basal IV became comparable to that achieved using a proved broiler diet. Two experiments were conducted using a depletion period followed by a supplemental period during which performance was measured on a paired-gain basis. Chicks were paired using gain during the depletion period as a criteria.

Growth of chicks receiving purified diets, which resulted in poor gains and abnormal feather development, gave inconsistent responses to corn oil and ethyl linoleate supplementation. Ethyl linoleate was used at one-half the level of corn oil to ascertain whether linoleic acid was responsible for growth responses found in the literature. Corn oil is approximately one-half linoleate.

Day-old chicks were yolkectomized in some experiments to remove fat reserves found in residual yolk sacs. These chicks usually gained commensurately with yolkectomized chicks receiving the low-fat basal.

Corn oil and abdominal hen fat were hydrogenated in some instances to determine whether fat alone or unsaturated fatty acids are the growth or gain promoting substances found in natural fats. Chicks gave responses to natural fats. Less response was recorded from hydrogenated materials, especially among yolkectomized chicks. Hydrogenated fats were less available to the chick as measured by fecal ether extractables.

Corn oil was fractionated into more and less saturated portions using the method of Newey et al. These fractions were added to the diet at a level based on yield in preparation and compared to corn oil supplementation. Responses in gain were noted from each fraction in proportion to the amount added indicating that growth promoting factors are found in equal concentration in each fraction.

Glycerol was added to some diets to make the diet less dusty and more palatable. Part of the response from adding liquid fats to the diet was thought to be due to increased palatability of the diet. Glycerol proved to be a growth depressant.

Digestive tracts of chicks fed purified diets were compared to those of chicks fed broiler diets. All portions of the tract showed underdevelopment when purified diets were fed, especially the gizzard and ceca.

Using the depletion and paired-gain supplemental period technique with a purified diet, 0.5 per cent corn oil gave gain increments comparable to 3.0 per cent corn oil.

Feed efficiencies were consistently improved when 1.0 per cent or more of natural fat was added to the otherwise adequate lot-fat Basal IV.

Body analyses of chicks from various lots indicated supplementation with 0.5 per cent fat slightly decreased moisture content and increased fat content of chicks as much as levels up to 3.0 per cent fat.