A STUDY ON THE CRITICAL NEED FOR FOLIC ACID IN PRACTICAL RATIONS FOR LAYING HENS AND BABY CHICKS

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INTRODUCTION

The synthesis of folic acid in 1945 by Angier and coworkers has opened a new field in nutritional research. Folic acid has been reported by investigators from all over the country to cure anemia in humans, rats and chicks as well as other species of animals.

Besides its anti-anemic properties, folic acid was found to promote normal growth and feathering in baby chicks fed an otherwise complete, purified diet. However, little research has been conducted along this line with practical rations for poultry. Therefore, investigation was undertaken to determine (1) the critical need for folic acid by laying pullets fed a diet composed only of practical ingredients in respect to egg production, fertility, hatchability and viability of progeny and (2) whether or not starting chicks require a dietary source of folic acid in such rations.

After materialization of this investigation, two independent papers were published by Taylor of California (1947) and by Schweigert and coworkers of Texas (1948) on the critical need for folic acid by laying hens and turkeys. In the California paper, a semi-practical diet was employed, and no mention was made of the progeny after hatchability data were assembled. The Texas workers included growth data of turkey poults from dams fed the basal diet with and without folic acid. Some research was done with laying pullets, but none on the progeny of these pullets fed the deficient diet.

Therefore, the major phase of this manuscript is to present the data obtained with progeny from pullets fed the unsupplemented and supplemented diets to observe the effect of maternal diet upon viability, growth, and feathering of progeny fed a diet composed only of practical ingredients.

REVIEW OF LITERATURE

The synthesis of pteroylglutamic acid by Angier et al. (1945) made possible rapid scientific progress in nutritional research for human beings and other species of the animal kingdom. During a period of 3 years (1945-1948) numerous publications pertaining to pteroylglutamic acid and related compounds have rendered the knowledge of pteroylglutamic acid very intricate and somewhat confusing. The interpretation of biological results of pteroylglutamic acid has become complicated by the existence of several naturally occurring derivatives of a parent molecule and by the variation which was encountered among different species of test organisms in response to their derivatives.

Jukes and Stokstad (1948) have done excellent research of summarizing the literature of pteroylglutamic acid and related compounds. Their extensive literature review is considered a foremost reference on this vitamin.

The complexity of the nutritional relationships of the pteroylglutamic acid family has been lessened somewhat by recent studies in the fields of enzymology and structural chemistry. Only a brief mention will be made of the history of the pteroylglutamic acid family in this manuscript.

The isolation of "folic acid" by Pfiffner, Binkley, Bloom, Brown, Bird, Emmett, Hogan and O'Dell (1943) and by Stokstad (1943) established the identity between Factor U of Stokstad and Manning (1938), vitamin B_c of Hogan and Parrott (1939) and Lactobacillus casei factor of Hutchings, Bohonos, Hegsted, Elvehjem and Peterson (1941).

Upon the isolation and synthesis of folic acid by Angier et al. (1945) the term pteroylglutamic acid was suggested for the new vitamin. This vitamin consists of a pteridine grouping linked through para-aminobenzoic acid to a single glutamic acid residue.

Before the synthesis of folic acid in 1945 by Angier and coworkers, the isolation of another form of folic acid by Hutchings, Stokstad, Bohonos and Slobodkin (1944) took place and was termed "fermentation Lactobacillus casei factor". The name pteroyltriglutamic acid was suggested for this fermentation L. casei factor by Angier et al. (1946) because this compound contained three glutamic acid groups.

The work of Binkley et al. (1944) showed that folic acid was bound up in a large molecule, forming a conjugate and that unless released by enzymatic process this folic acid conjugate was not available to either Lactobacillus casei or Streptococcus lactis R. Yeast concentrates rich in folic acid activity, produced very little growth for these two microorganisms. However, when these yeast concentrates underwent enzymatic digestion, folic acid was released and became active for both microorganisms. This factor was designated vitamin B_c conjugate by Bird, Binkley, Bloom, Emmett and Pfiffner (1945) and isolated by Pfiffner et al. (1945) who also stated that vitamin B_c conjugate was active for the chick. The term pteroyhexaglutamylglutamic acid was suggested for vitamin B_c conjugate by Pfiffner, Calkins, Bloom and O'Dell (1946) due to the fact that seven glutamic acid groups were involved. Jukes and Stokstad (1948) substituted the term pteroylheptaglutamic acid in preference to the longer name of Pfiffner et al. (1946).

Pteroic acid, synthesized by omitting glutamic acid, is considered an "incomplete" molecule. It consists of a pteridine grouping linked to para-aminobenzoic acid and is biologically active for Streptococcus lactis R but not for Lactobacillus casei or for chicks or rats.

In the meantime, the existence of 2 new vitamins, B_{10} and B_{11}, distinct from folic acid, was reported by Briggs, Luckey, Elvehjem and Hart (1943, 1944, 1945), to be required by the chick for feathering and for growth, respectively. However, Luckey, Moore, Elvehjem and Hart (1946 a) showed that vitamins B_{10} and B_{11} did not produce a supplementary effect in the presence of synthetic folic acid. These results presumably suggest that vitamins B_{10} and B_{11} represented a conjugate or conjugates of pteroylglutamic acid.

Thus it can be seen that interpretation of biological results of the different members of the pteroylglutamic acid family and their nutritional relationships for different species of test organisms has become extremely difficult.

The synthesis of synthetic pteroylglutamic acid, referred to as folic acid heretofore, has enabled the physician to administer this vitamin to human patients suffering from various diseases. It is beyond the scope of this manuscript to discuss the extensive literature on folic acid in human medicine. Jukes and Stokstad (1948) and Berry and Spies (1946) have presented an excellent summary of the therapy and clinical effects of folic acid. Human diseases, including Addisonian pernicious anemia, sprue, nutritional macrocytic anemia, celiac disease, glossitis of pellagra, and others, have been reported to respond to folic acid administration. The synthesis of folic acid has proved to be a boon to the medical world because many patients are allergic to liver extract therapy when treated for various anemias.

In regard to folic acid assays, microbiological and biological assays are most commonly employed by nutritionists and biochemists. A variety of methods for the microbiological assay of folic acid has been proposed by a number of research workers such as Bird, Bressler, Brown, Campbell, and Emmett (1945), Luckey, Briggs, and Elvehjem (1944), Tepley and Elvehjem (1945) and others.

The 2 organisms used for assay purposes are Streptococcus lactis R and Lactobacillus casei. The former has the advantage of giving more reproducible results and of requiring shorter incubation periods than with the latter organism. However, the former requires approximately 5 times as much folic acid as the latter to give half maximum growth, thereby limiting the assay of very low-potency materials.

The chick assay appears to express the total folic acid content of a sample including both the free and conjugated forms, as reported in the work of Bird, Bressler, Brown, Campbell and Emmett (1945) and Lillie and Briggs (1947 a).

The use of rats and monkeys for assay animals has been described in detail by Day and Totter (1947).

Luckey, Briggs, Moore, Elvehjem and Hart (1945) compared a variety of chemical and enzymic methods for the liberation of folic acid. Taka-diastase, chicken pancreas, hog kidney conjugase, autoclaving, and others were some methods mentioned.

The role of folic acid in poultry nutrition is a very interesting, and also a very important, one. The biological activity of crystalline pteroylglutamic acid for the chick was first reported by Pfiffner, Binkley, Bloom, Brown, Bird, Emmett, Hogan and O'Dell (1943). Their "vitamin B_c" obtained from liver was fed at a level of 250 micrograms per 100 grams of diet which prevented anemia and promoted good growth in chicks.

A level of 40 micrograms per 100 grams of diet was found by Campbell, Brown and Emmett (1944 a) to be sufficient for normal hemoglobin, hematocrit, red cell count and thrombocyte values. However, the authors concluded that 400 micrograms per 100 grams of diet were required for the production of normal leucocyte levels in chicks.

Pfiffner, Calkins, O'Dell, Bloom, Brown, Campbell, and Bird (1945) found that a level of 25 micrograms of pteroylglutamic acid, either free or as the conjugate, per 100 grams of diet resulted in maximum growth with submaximal hemoglobin response.

Pteroylglutamic acid at the level of 500 micrograms per 100 grams of purified diet was essential for normal feather development in chicks according to Oleson, Hutchings and Sloane (1946). The effect was not lessened by adding sulfamerazine or certain other "intestinal antiseptics".

Hutchings, Oleson and Stokstad (1946) stated that maximum growth and hemoglobin values in chicks fed on a purified diet was made possible when pteroylglutamic acid was added at the rate of 50 to 100 micrograms per 100 grams of diet. Luckey, Moore, Elvehjem and Hart (1946 a) claimed that chicks required 25 micrograms of folic acid per 100 grams of purified diet for growth, feathering, and hemoglobin formation. When sulfasuxidine was added to the diet, the requirement was increased to a level between 50 and 100 micrograms per 100 grams of diet. On the other hand, the folic acid requirement of chicks was found not to be increased by the addition of sulfasuxidine to a purified diet, according to Robertson, Daniel, Farmer, Norris and Heuser (1946).

Daniel, Farmer and Norris (1946) noticed evidence of perosis in chicks on a purified diet without folic acid. The incidence was increased by adding 2 percent of sulfasuxidine. Perosis was prevented by the addition of 20 and 30 micrograms of folic acid in the diet without and with sulfasuxidine, respectively. A higher incidence of perosis on a sulfasuxidine-containing purified diet led the authors to conclude that folic acid stimulated intestinal flora to produce an unknown anti-perotic factor.

A relationship between a folic acid deficiency in chicks and an absence of normal response to administration of diethylstilbestrol was reported by Hertz and Sebrell (1944). Later work by Hertz (1945) showed a direct quantitative relationship between the degree of oviduct response to stilbestrol and the dietary level of folic acid. These findings were confirmed by Haque (1948).

Haque, Lillie, Shaffner and Briggs (1948) suggested that folic acid be included in a purified diet to counteract the toxic effects of the thyroxine injections in New Hampshire chicks.

A comparison of injection versus oral administration of folic acid in the chick revealed very slight, if any, differences from the standpoint of growth, according to Campbell, Brown and Emmett (1944 b). However, Frost and Dann (1946) found that the vitamin was approximately twice as effective by injection. This finding was confirmed by Robertson, Fiala, Scott, Norris and Heuser (1947) who stated that chicks deprived of folic acid the first 4 weeks of life responded to a single administration of folic acid given either orally or intramuscularly and that the injection method was more than twice as effective as the same amount administered orally.

Scott, Norris, Heuser and Bruce (1945) found that conjugated folic acid at the level of 50 micrograms per 100 grams of a purified diet resulted only in partial effectiveness in promoting growth and preventing anemia in chicks. However, when the lactone of either 5-pyridoxic acid or 4-pyridoxic acid was added at a level of 50 micrograms per 100 grams of a purified diet together with folic acid fed at the same level, a marked gain in weight was produced and anemia was completely prevented. Hens on a commercial diet were bled by Scott, Norris and Heuser (1946) to induce anemia. The rate of regeneration of hemoglobin was followed, and injections of either 4-pyridoxic acid or folic acid at the level of 50 micrograms daily hastened the rate of hemoglobin formation. When both supplements were administered together, the rate was further increased.

Folic acid was found by Scott, Norris, Charkey, Daniel and Heuser (1946) to be effective against anemia in chicks without the addition of pyridoxic acid lactone to the diet. On the other hand, Petering, Marvel, Glauser, and Waddell (1946) showed evidence with chicks that 4-pyridoxic acid was supplementary on the utilization of pteroyltriglutamic acid. Jukes and Stokstad (1947) reported that pteroyltriglutamic acid without pyridoxic acid was as effective as pteroylglutamic acid on a molar basis in promoting growth and preventing anemia in chicks. As compared with pteroylglutamic acid, pteroyltriglutamic acid appeared to be utilized completely with or without addition of 4-pyridoxic acid by chicks.

Pteroylheptaglutamic acid was utilized as efficiently as pteroylglutamic acid without the addition of pyridoxic acid by chicks fed a purified diet, according to Binkley, Bird, Bloom, Brown, Calkins, Campbell, Emmett and Pfiffner (1944).

Jukes and Stokstad (1946 a, 1946 b) were the first to report that normal production and reproduction in chickens could take place on a purified diet containing pure vitamin B complex factors including folic acid. Reproduction in 2 generations of chickens on a purified diet was reported by Jukes, Stokstad and Gilbert (1948).

Folic acid was found to prevent depigmented feathers of Black Leghorn chicks fed a purified diet, when injected at the rate of at least 10 micrograms per day, according to Frost, Dann and McIntire (1946). Growth and feathering were in proportion to the level of folic acid used.

Lillie and Briggs (1947 b) stated that the strain of New Hampshire chicks used required a minimum of 150 micrograms of folic acid per 100 grams of a purified diet for optimum growth and 200 micrograms per 100 grams of the same diet for normal feathering. Folic acid was found to be necessary for the prevention of abnormal feather pigmentation in New Hampshire chicks fed synthetic diets, according to Lillie and Briggs (1947 c).

The turkey poult developed a spastic type of cervical paralysis when fed a purified diet deficient in folic acid, as reported by Richardson, Hogan and Kempster (1945). This paralysis was counteracted by folic acid administration. These observations were confirmed by Jukes, Stokstad and Belt (1947).

Russell, Taylor and Derby (1947) estimated the folic acid requirement of the turkey poult to be 200 micrograms per 100 grams of a synthetic diet. This level of folic acid was required to prevent cervical paralysis at the 25 and 43 percent protein levels of the purified ration. At a level of 200 micrograms of folic acid per 100 grams of diet, the 43 percent protein level produced better feathering than the 25 percent protein level.

Lance and Hogan (1948) suggested that a level of 200 micrograms of folic acid per 100 grams of synthetic diet was required by the turkey poult for maximum growth and normal development.

Schweigert, German, Pearson and Sherwood (1948) showed no differences in egg production, hatchability, hemoglobin level or general appearance in turkeys or laying pullets when fed a basal diet low in folic acid (42 micrograms per 100 grams of diet) as compared with those fed the basal diet supplemented with folic acid at the level of 200 micrograms per 100 grams of diet. However, the folic acid content of eggs laid by turkeys and pullets was much lower in the basal group than in the folic acid supplemented group. Egg yolks contained greater quantities of folic acid than albumen. The folic acid conent of blood observed after enzymatic digestion of the blood samples was shown to be approximately the same for both groups of chickens and turkeys, regardless of dietary treatment. Turkey poults and turkey hens fed the basal diet exhibited a higher mortality and a slower growth rate than the supplemented groups. Poults from dams fed 200 micrograms of folic acid per 100 grams of basal diet required 80 micrograms of folic acid per 100 grams of growing diet, whereas those from dams fed the basal diet required more than 80 micrograms of folic acid per 100 grams of diet.

Yearling White Leghorn hens fed a semi-purified diet low in folic acid (not in excess of 12 micrograms per 100 grams of diet) required a level of folic acid greater than that in the basal diet for normal hatchability, according to the work of Taylor (1947). The optimal level for normal hatchability was not determined. The folic acid requirement for egg production was low on this diet.

In spite of the extensive research on the importance of folic acid and its requirements in poultry nutrition, Luckey, Moore, Elvehjem and Hart (1946 b) concluded that no definite requirement for folic acid in nutrition could be established because the response to a given amount of folic acid depended upon the type of ration used. For example, high fat diets or diets containing glucose, sucrose, or starch as the sole carbohydrate resulted in a lower growth response than those diets containing high protein, low fat, or corn meal and dextrin as the carbohydrates when folic acid was incorporated in the diets. Hemoglobin responses were better on diets containing dextrin or corn meal than diets containing other carbohydrates.

It is beyond the scope of this manuscript to discuss in detail the literature on the role of folic acid in the nutrition of other species commonly used as assay organisms in the nutritional laboratory. However, only a very few references will be cited to reveal the importance of folic acid in the nutrition of other species.

Folic acid requirements of microorganisms have been investigated by Stokes and Larsen (1945). That the rat could utilize conjugated pteroylglutamic acid was shown by Wright, Skeggs, Welch, Sprague and Mattis (1946). Nielsen and Black (1944) indicated that mice fed a purified diet containing sulfasuxidine required folic acid. The "lactation performance" of mice on purified diets as measured by percentage and size of litters weaned was increased by addition of a concentrate containing pteroylglutamic acid, as reported by Cerecedo and Mirone (1947).

Folic acid was needed by guinea pigs (Woolley and Sprince, 1945) and by mink (Schaefer, Whitehair and Elvehjem, 1946) to prevent loss of body weight, diarrhea, lethargy, terminal convulsions and death.

Krehl, Torbet, de la Huerga and Elvehjem (1946) observed that dogs fed a purified diet deficient in niacin responded to folic acid administration.

Krider, Van Poucke, Becker and Carroll (1946) showed evidence that sows fed a folic acid concentrate produced more vigorous and thriftier pigs than sows fed the basal diet. An inter-relationship of folic acid and biotin was established in the nutrition of swine by Cunha, Colby, Bustad and Bone (1948).

Folic acid was needed for growth for the larvae of the mosquito "Aedes aegyptii" (Golberg, de Meillon and Lavoipierre, 1945), and for the larvae of flour moth and the meal worm (Fraenkel and Blewett, 1946).

In the biological and microbiological assays for folic acid activity in foods and common feedstuffs, the interpretation of such results become a difficult one because of the wide variations in animal and microorganism assay techniques which render interpretation of the results in terms of pteroylglutamic acid extremely complicated.

Folic acid exists in the form of conjugates, most of which are inactive in the microbiological assays until treated with certain enzyme preparations which liberate folic acid, as is shown in the work of Bird, Binkley, Bloom, Emmett and Pfiffner (1945). Regardless of the type of enzyme preparation, folic acid values vary. Thus, the presence of inhibitors of enzyme system in natural foods is brought to attention by Swendseid, Bird, Brown and Bethell (1947).

Folic acid showed the greatest losses due to autoclaving of all the vitamins of the B complex in nautral foods, according to the work of Cheldelin, Woods and Williams (1943) who concluded that the vitamins might become "bound" in tissues during autoclaving. These results were confirmed by Schweigert, Pollard and Elvehjem (1946) who found that as much as 92 percent of folic acid in meats was destroyed by cooking.

The storage, retention and distribution of folic acid in the chick was described by Moore, Lepp, Luckey, Elvehjem and Hart (1947). High oral intakes of folic acid were stored inefficiently in chick livers of day-old White Leghorn chicks fed a folic acid-deficient synthetic diet. The folic acid content in chick muscle tissue was not affected by the level of oral intake of folic acid. Those chicks given large oral doses of folic acid and then placed on the deficient diet grew as well as those receiving an equivalent amount of folic acid by injection. The vitamin was distributed in greatest quantities in the liver and kidney and in least quantities in the muscle and skin.

Lillie and Briggs (1947 a) determined the folic acid activity of common feedstuffs by means of a biological assay with the chick. Cereal grains, grain by-products and dairy by-products were relatively poor sources of folic acid. Soybean oil meal, alfalfa meal and fermentation solubles were relatively good sources; yeast from several origins and liver meals were excellent sources. A sample of menhaden fish meal did not show any folic acid activity.

EXPERIMENTAL PROCEDURE AND RESULTS

A. Studies with Laying Pullets

1. General experimental procedure. In the first of 2 experiments conducted with laying pullets, New Hampshire day-old chicks hatched in March, 1947, from eggs laid at the College Poultry Farm were distributed into 4 uniform groups of 20 chicks each. The chicks were individually wing-banded and reared in electrically heated batteries with raised screen floors. Feed and water were supplied *ad libitum*. Individual weighings were made weekly up to and including the 16th week of age.

Group I received a practical ration (diet 1) designed to be low in folic acid. Groups II and III received this basal diet with synthetic folic acid supplemented at the rate of 200 and 400 micrograms per 100 grams of diet, respectively. Group IV received a typical broiler mash (diet 4).

The males were removed at 9 weeks of age. At 16 weeks of age when the pullets were transferred from growing chick batteries to a 60 bird conventional-type laying battery, the number of pullets per group was 9, 11, 6, and 8, respectively. Farm-raised pullets of the same hatch as the battery-raised pullets were used to fill up the laying battery to make a total of 15 pullets per group. Groups I, II, and III continued to receive their respective diets, whereas Group IV received the Maryland all-purpose laying mash (diet 3). Crushed oyster shell was fed twice weekly.

Beginning at the 16th week of age, the pullets were weighed on the first of each month. Daily egg production records and monthly over-all feed consumption records were kept. Artificial insemination according to the method described by Quinn and Burrows (1936) was performed twice weekly with the pooled semen of several New Hampshire cockerels maintained in batteries. Eggs were pedigreed daily and stored in a refrigerator at 60°F until setting time. Settings were made weekly during the fall of 1947, at 10-day intervals during the winter and spring months of 1947-1948, and finally at 2-week intervals during the summer of 1948. On the twenty-second day all the chicks that hatched were wing-pedigreed and placed on experiments to be discussed in detail in a later paragraph. Those eggs that did not hatch were broken out, and time of embryonic mortality was determined.

In the second experiment conducted with laying pullets, 16 New Hampshire pullets 10 months old were used. Prior to selection in February 1948, these pullets had been maintained on the Maryland all-purpose laying mash in laying batteries. At the time of selection these pullets were placed on basal diet 2 containing ground wheat instead of wheat middlings for a 4-week depletion period to deplete, if any, folic acid reserves. At the end of the depletion period, the pullets were divided into 2 groups of 8 each. The first group (Group V) was continued on basal diet 2; the second group (Group VI) received this basal diet 2 supplemented with 200 micrograms of synthetic folic acid per 100 grams of diet. Subsequently, the experimental procedure was precisely the same as that described in the first experiment, except that chicks were not saved for further experiments.

2. Experimental diets. The basal diets 1 and 2 (Table I) for experiments 1 and 2 with laying pullets were designed to be very low in folic acid activity. On the basis of folic acid activity of feedstuffs (Lillie and Briggs, 1947 a), the basal diet containing wheat middlings (diet 1) for Experiment 1 and ground wheat (diet 2) for Experiment 2 contained a folic acid activity of 33.8 and 26.3 micrograms per 100 grams of diet, respectively.

The Maryland all-purpose laying mash (diet 3) and the Maryland broiler ration (diet 4) shown in Table I contained 164.2 and 159.5 micrograms, respectively, of folic acid per 100 grams of ration.

Since cereal grains contained traces of folic acid, the formulating of a practical ration devoid of folic acid was not possible. For this reason, the basal diets under study were formulated so that the folic acid activity of the diet was as low as possible without greatly curtailing the balance of other nutritional requirements in the ration. Because of their high folic acid activity, alfalfa leaf meal and soybean oil meal were omitted from the diet. Meat scrap was not included because the folic acid activity of this material was not known. The experimental diets were mixed on the College Poultry Farm. Precautious were made with great care to prevent contamination of the experimental diets, so that the folic acid activity of such diets would not be increased by slight traces of alfalfa leaf meal and soybean meal, both of which are known to be high in folic acid activity. Five hundred pounds of feed were mixed at a time about every 4 weeks. For each group of pullets in Hen Experiments 1 and 2, 50 pounds were supplied at a time.

The synthetic folic acid obtained from Lederle Laboratories was added in dry form to a small handfull of ground yellow corn and mixed thoroughly. This pre-mix was then added to the basal diet.

3. Hen Experiment 1. To determine the critical need for folic acid by laying pullets fed a folic acid-low practical ration, this experiment was set up for a period of 11 months. Table II represents a summary of egg production and feed consumption. The average egg production per hen

TABLE I

COMPOSITION OF DIETS

INGREDIENT	DIET 1	DIET 2	DIET 3	DIET 4	FOLIC ACID ACTIVITY
	%	%	%	%	Mcg./gram
Ground yellow corn	26	26	25.25	20	0.3
Ground heavy oats	30	30	12.5	15	0.2
Ground wheat	--	15	--	--	0.4
Wheat middlings	15	--	10.0	15	0.9
Wheat bran	--	--	12.5	12.5	2.1
Soybean oil meal	--	--	10.0	10.0	7.7
Corn gluten meal	10	10	5.0	7.5	0.2
Alfalfa leaf meal	--	--	5.0	5.0	6.0
Fish meal	7.5	7.5	5.0	5.0	0.0
Meat scrap	--	--	5.0	5.0	--
Dried whey	--	--	5.0	2.5	0.9
Dried skim milk	7.5	7.5	--	--	0.6
Fermentation solubles (80 mcg. riboflavin/gm)	--	--	1.25	--	5.1
Oyster shell flour	2.0	2.0	2.0	1.25	--
Rock phosphate defluorinated	1.0	1.0	0.75	0.5	--
Salt, iodized	0.5	0.5	0.75	0.5	--
Vitamin A and D oil (400 units D/gram)	0.5	0.5	0.5	0.2	--
Mn SO_4	0.012	0.012	0.012	0.012	--
Riboflavin, synethetic	150 mg per 100 lbs		--	--	--
Nicotinic acid	2 grams per 100 lbs		--	--	--
Folic acid mcg. per 100 grams	33.8	26.3	164.2	159.5	--
Percent protein, calculated	18.4	18.1	20.4	21.6	

TABLE II

EFFECT OF DIET UPON EGG PRODUCTION AND FEED CONSUMPTION OF NEW HAMPSHIRE PULLETS IN EXPERIMENT 1

DIET*	TOTAL BIRD DAYS (Aug 1 to July 1)	TOTAL EGGS LAID	AVERAGE EGG PRODUCTION PER HEN	TOTAL FEED CONSUMPTION LBS.	AVERAGE FEED CONSUMPTION PER HEN LBS.	POUNDS OF FEED PER DOZEN OF EGGS
Group I	4,159	1,823	146.8	1188.5	95.7	7.9
Group II	3,839	1,088	94.9	1036.5	90.4	11.4
Group III	4,303	1,585	123.4	1168.5	90.9	8.9
Group IV	4,167	1,502	120.8	1221.5	98.2	9.8

* Group I - Diet 1
Group II - Diet 1 + 200 mcg. folic acid per 100 grams of diet
Group III - Diet 1 + 400 mcg. folic acid per 100 grams of diet
Group IV - Diet 3

was greater, and the number of pounds of feed required per dozen of eggs was less, in Group I than in the 3 other groups. These results indicate clearly that a practical ration containing no vegetable protein supplement and containing only 33.8 micrograms of folic acid per 100 grams of diet can sustain normal egg production and also normal hatchability, as is shown in Table III. In spite of the highest hatchability being obtained in Group IV, the differences in the hatchability data of the 4 groups of dams were so slight to indicate a dietary influence upon hatchability. The fertility data revealed no apparent differences, even in Group III because

TABLE III

EFFECT OF DIET UPON FERTILITY AND HATCHABILITY OF NEW HAMPSHIRE PULLETS IN EXPERIMENT 1 *

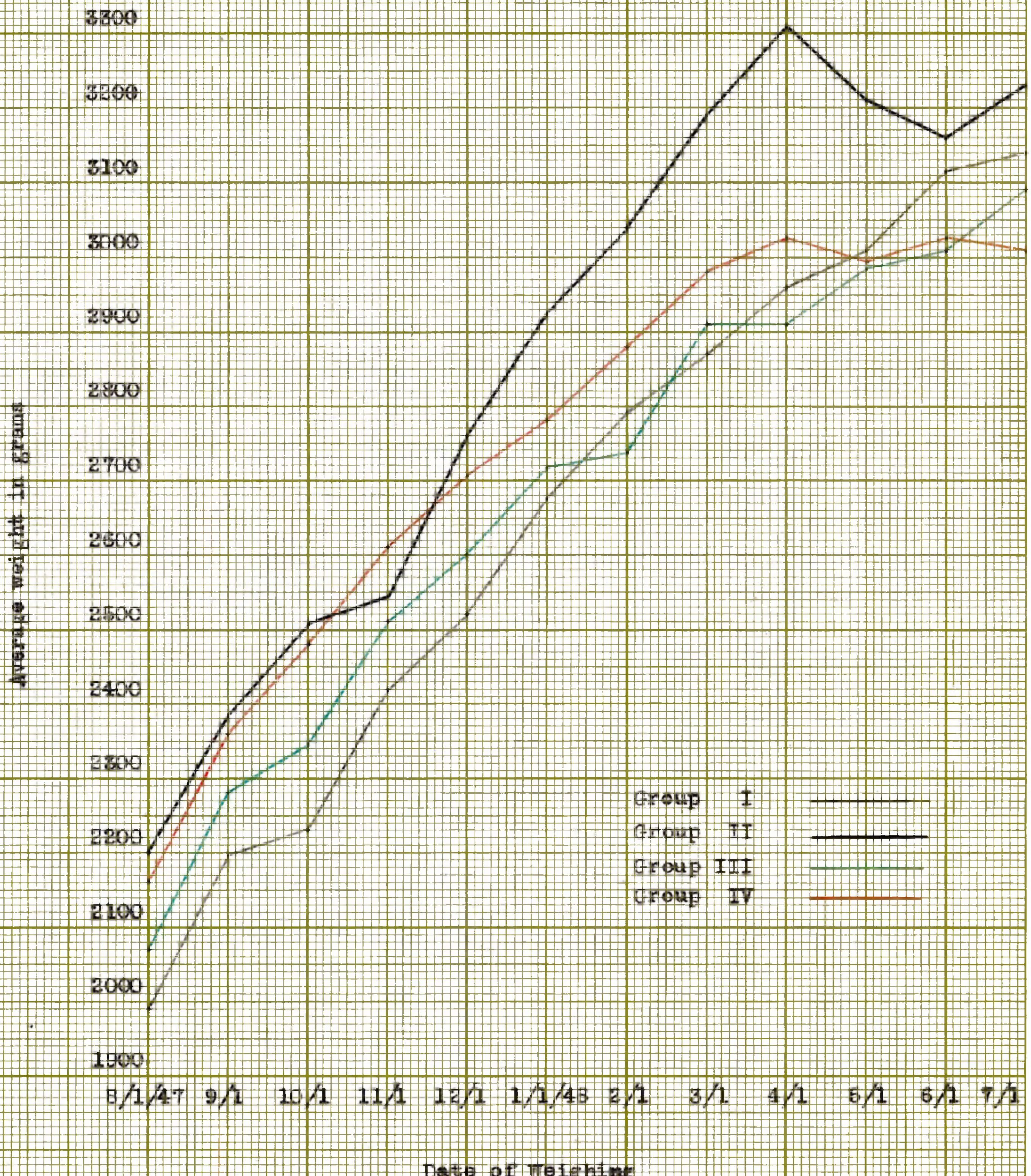
DIET**	NO. EGGS SET	PERCENT FERTILITY	PERCENT HATCHABILITY OF FERTILE EGGS
Group I	898	92.3	78.4
Group II	459	91.9	78.6
Group III	721	84.3	77.9
Group IV	711	90.3	79.9

* For complete details, see Appendix, page 78
** Diets are described in Table II

the percent fertility for this group would have been raised to 89.8 percent, if 2 dams in Group III had been eliminated due to their poor fecundity. At hatching time, all the progeny from the 4 groups of dams appeared normal with respect to down color, size, weight, and viability.

Chart I indicates that synthetic folic acid added at the level of 200 micrograms per 100 grams of basal diet will support body weight maintenance,

Chart 1. Effect of Diet Upon Body Weight of New Hampshire Pullets in Experiment 1.



as compared with Group I. The mean body weight of Group I dams was the lowest of the 4 groups from August 1947 through March 1948 except in February in which Group I was heavier than Group III. Throughout the entire experimental period Group II dams ranked heaviest, except on November first when these dams were second to Group IV dams in weight. Group IV dams were the second heaviest from August to April. The high mean body weight characteristic of Group II may possibly explain one of the reasons for poor egg production in this group.

Hemoglobin studies with the 4 groups of dams showed some differences, as shown in Table IV. The method for hemoglobin determinations was described in detail by Evelyn (1936). On both dates the number of grams of hemoglobin per 100 cc. of whole blood was the lowest in Group I dams. The addition of synthetic folic acid to the basal ration apparently increased the hemoglobin level per 100 cc. of whole blood to approximately the same level as that obtained on the Group IV diet. In all instances, the hemoglobin level was higher in the second than in the first test.

TABLE IV

HEMOGLOBIN STUDIES WITH NEW HAMPSHIRE PULLETS
IN EXPERIMENT 1

DATE	GROUP I	GROUP II	GROUP III	GROUP IV
September 1, 1947	8.23	9.14	8.61	8.90
July 25, 1948	9.45	9.91	9.75	9.74

Biological determinations of the folic acid activity of egg yolks from eggs laid by the 4 groups are summarized in Table V. The chick assay used for expressing the total folic acid content of the sample including both the free and conjugated forms is explained in detail by Lillie and

TABLE V

FOLIC ACID ACTIVITY OF EGG YOLKS FROM EGGS LAID BY NEW HAMPSHIRE PULLETS IN EXPERIMENT 1

SUPPLEMENT TO BASAL DIET 110	EXPERIMENT A			EXPERIMENT B		
	NO. CHICKS		AVERAGE WEIGHTS AT 4 WEEKS	NO. CHICKS		AVERAGE WEIGHTS AT 4 WEEKS
	AT START	AT 4 WEEKS	(gms.)	AT START	AT 4 WEEKS	(gms.)
None	10	1	58.0	10	4	100.5
25 mcg. folic acid/100 grams	10	8	225.2	10	9	266.6
50 " " " " "	10	8	205.4	10	8	290.1
100 " " " " "	10	9	226.4	10	9	280.6
200 " " " " "	10	6	279.2	10	10	278.5
1 egg yolk (Group I)/kilogram	10	4	95.3	--	--	--
2 " " (Group I) "	10	6	135.8	10	4	109.0
2 " " (Group II) "	10	5	151.2	10	4	163.0
2 " " (Group III) "	10	2	158.5	10	2	146.0
2 " " (Group IV) "	10	2	202.5	10	3	137.3

Briggs (1947 a). None of the chicks fed egg yolks, regardless of source, grew as well as those fed 25 micrograms of folic acid per 100 grams of diet, a fact indicating that none of the egg yolks contained a folic acid activity of more than 25 micrograms per 100 grams. The values for the folic acid activity by means of a biological assay are conflicting in Experiments A and B (Table V) due to the fact that a disease epidemic of a mild nature occurred in the laboratory. For this reason, no attempt is made to determine the actual folic acid activity of egg yolks from eggs laid by the 4 groups. Nevertheless, it is interesting to observe that in both chick experiments, the growth rate of chicks fed egg yolks from Group II dams was greater than that of chicks fed egg yolks from Group I dams. This finding indicates a difference in the folic acid activity of egg yolks from Group I dams and Group II dams fed the basal diet and 200 micrograms of folic acid per 100 grams, respectively.

4. Experiment 2. When ground wheat became available, a second experiment was set up to make a comparison of two feedstuffs differing in their folic acid activity upon egg production, fertility, hatchability, and weight maintenance. Tables VI and VII show some differences in egg production and hatchability in favor of Group VI fed the supplemented diet. The number of pounds of feed required to produce a dozen of eggs was less on the Group VI diet. These results may indicate that the basal diet containing ground wheat with a total folic acid activity of 26.3 micrograms per 100 grams of diet requires additional folic acid to sustain normal egg production and hatchability, as compared with a folic acid-low ration containing wheat middlings. No differences in the fertility and general appearance of progeny were observed.

TABLE VI

EFFECT OF DIET UPON EGG PRODUCTION AND FEED CONSUMPTION OF NEW HAMPSHIRE PULLETS IN EXPERIMENT 2

DIET*	TOTAL BIRD DAYS (March 13 to July 30)	TOTAL EGGS LAID	AVERAGE EGG PRODUCTION PER HEN	TOTAL FEED CONSUMPTION	AVERAGE FEED CONSUMPTION PER HEN	POUNDS OF FEED PER DOZEN OF EGGS
Group V	1,120	465	58.1	294.5	36.9	7.6
Group VI	1,120	542	67.8	293.3	36.6	6.5

* Group V - Diet 2
Group VI - Diet 2 + 200 mcg. folic acid per 100 grams of diet.

TABLE VII

EFFECT OF DIET UPON FERTILITY AND HATCHABILITY OF NEW HAMPSHIRE PULLETS IN EXPERIMENT 2*

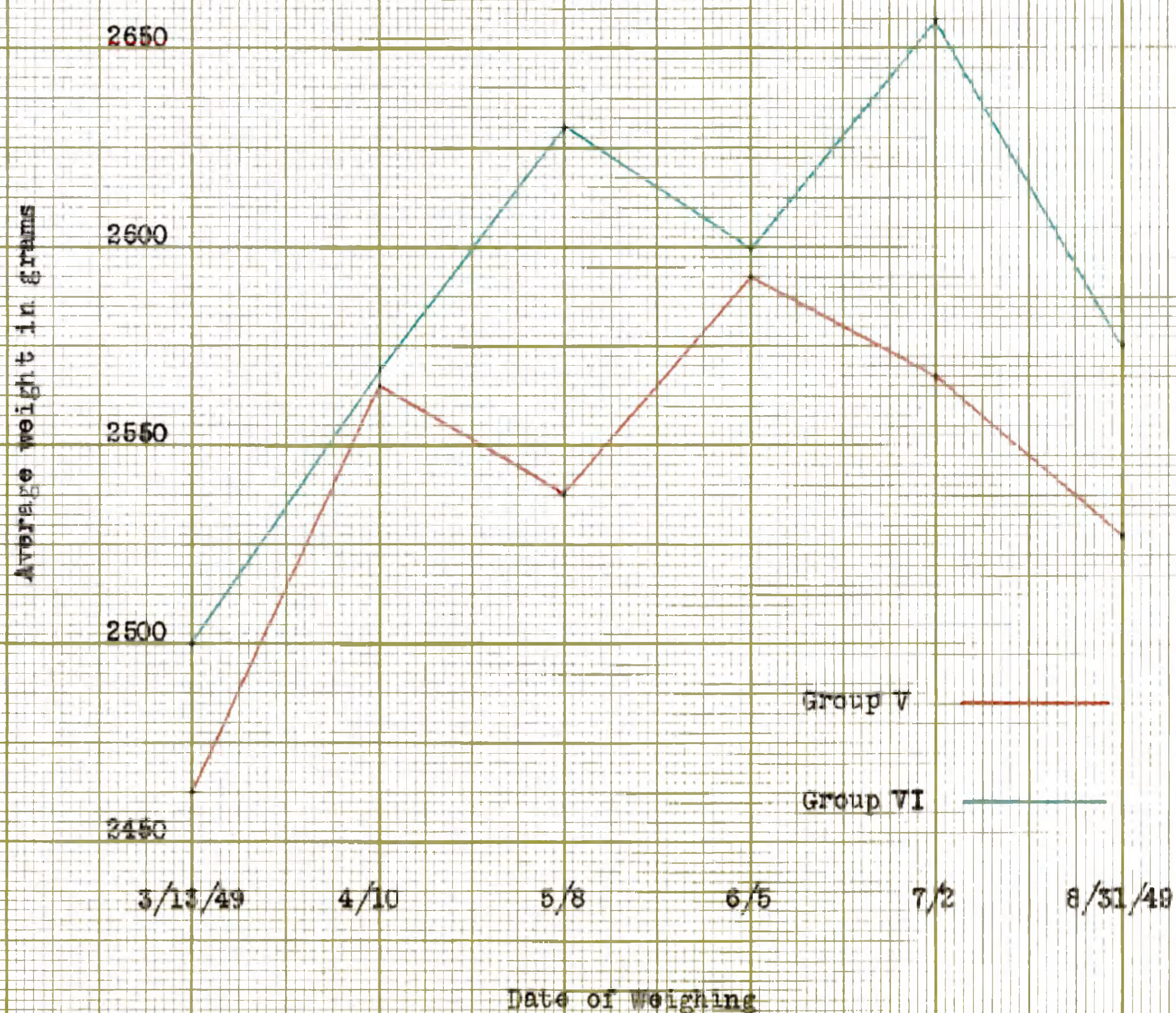
DIET**	NO. EGGS SET	PERCENT FERTILITY	PERCENT HATCHABILITY OF FERTILE EGGS
Group V	459	88.7	69.5
Group VI	527	90.7	83.9

* For complete details, see Appendix, page 79
** Diets are described in Table VI

Throughout the 140-day experimental period Group VI dams were heavier than Group V dams, as is shown in Chart II.

5. <u>Summary of laying pullet experiments</u>. When pullets were fed a practical ration which contained wheat middlings and which also contained 33.8 micrograms of folic acid per 100 grams of diet, egg production was highest on the basal diet. No apparent differences in fertility and hatchability were noticed among the 4 diets. Body weight data, hemoglobin studies, and folic acid assays of egg yolks, all clearly show a difference between Group I fed the basal diet and the 3 other groups. When ground wheat was substituted for wheat middlings, the total folic acid activity of the basal ration was lowered to 26.3 micrograms per 100 grams of diet. Consequently, a wide difference was noted in egg production, hatchability, and body weights in favor of Group VI fed 200 micrograms of folic acid per 100 grams of diet. The fertility data indicated no apparent differences between the 2 groups. In both Experiments 1 and 2 all the progeny appeared normal with respect to down color, size, weight, and viability.

Chart 2. Effect of Diet Upon Body Weights of New Hampshire Pullets in Experiment 2.



B. Studies with Chicks

1. General experimental procedure. All chick studies were divided into 3 main experiments; namely, Chick Experiment 1, which was devoted to pedigreed chicks obtained from pullets in Hen Experiment 1 described in a previous paragraph; Chick Experiment 2, which dealt with pedigreed chicks obtained from Group I dams in Hen Experiment 1; and Chick Experiment 3, which was devoted to chicks obtained from mass matings on the College Poultry Farm where a complete breeder mash was fed. In these 3 experiments, all chicks were maintained in electrically-heated metal batteries with raised screen floors. Feed and water were supplied *ad libitum*. At the termination of the individual experiments all survivors were sexed by the comb method, unless otherwise indicated, and examined carefully for any effects of the maternal diet as well as the chick diet upon feather pigmentation and structure. Those chicks showing abnormal black and/or white areas in the feathers were given the symbol BW. Those chicks showing poor feather structure were given the symbol B_{10}, and all the remaining chicks showing normal feather pigmentation and structure were given a score for the degree of feathering. Table VIII gives the system employed in determing the feather score.

TABLE VIII

SYSTEM USED IN FEATHER SCORING

ADJECTIVE SCORE	NUMERICAL SCORE
Very Poor	0
Poor	25
Fair	50
Good	75
Very Good	90
Excellent	100

Body weights were recorded at 2-week intervals, unless otherwise indicated. In all chick experiments the mean body weights included in the tables contained in the following pages represent only those weights of survivors at the termination of the experiments. In other words, the weights of chicks that died during the experimental periods were not included for final averaging. The final averages for each weight class (such as hatching time, 2 weeks, 4 weeks, 6 weeks, and 8 weeks) were not determined until after the termination of the experiments when the survivors were sexed.

2. Experimental diets. The same diet as used for laying pullets (basal diet 1) was used as the basal diet for all chick experiments. The diets employed in Chick Experiment 1 may be designated as treatments A, B, and C. Treatment A refers to the feeding of the basal diet throughout the 8-week period; treatment B refers to the feeding of the basal diet during the first 4 weeks followed by diet 4 (Table I) during the second 4 weeks; treatment C refers to the feeding of diet 4 throughout the 8-week period. The first pedigreed hatch of chicks obtained from the 4 groups of dams in Hen Experiment 1 was placed on treatment A. The following 12 hatches, in groups of 3 hatches each, were alternated on treatments B and C, respectively. Finally, the first of the last 2 hatches was placed on treatment B and the other on treatment C. Altogether, 15 hatches were used on treatments A, B, and C. The reason for placing only one hatch on treatment A was because dried skim milk was available only in limited quantities.

In Chick Experiment 2 only two diets per hatch were used throughout a series of 10 hatches. One diet was the hen basal diet 1, and the other diet included a supplementation to the hen basal diet 1 to observe its effects upon growth, feather pigmentation, and feather structure.

Different levels of synthetic folic acid were supplemented to the basal diet 1 in Chick Experiment 3. A sample of Parvo, a crude folic acid supplement, was tested for its nutritional value in a practical ration low in folic acid. The label description of Parvo is summarized in Table IX. Both synthetic folic acid and Parvo were added in dry form to a small batch of the basal diet and mixed very thoroughly before being incorporated into the basal diet.

TABLE IX

COMPOSITION OF PARVO

INGREDIENT	PERCENT
Folic acid-not less than	3.0
Inactive pterins	23.0
NaCL-not more than	0.9
Diatomaceous earth	68.0
Moisture-not more than	5.0

3. Chick Experiment 1. This experiment was conducted to determine the effect of maternal diet upon mortality, growth rates, and feather pigmentation and feather structure of progeny. Mortality data, as given in Table X, revealed that the mortality was greatest in the progeny of Group I dams, irrespective of chick diet. The mortality of the progeny of Group II dams was nearly equivalent to that obtained with progeny of Group IV dams, thereby indicating that the addition of synthetic folic acid to the maternal diet is required to prevent high mortality in the progeny. No explanation for the greater mortality rate of chicks from Group III dams than from Group II dams on treatments B and C is given.

A comparison of the 3 treatments will reveal an influence of the chick diet upon the mortality of progeny. For progeny from dams fed Group I and II diets, the mortality was lowest in chicks on treatment C than in those

TABLE X

EFFECT OF MATERNAL DIET UPON THE MORTALITY OF PROGENY

MATERNAL DIET	TREATMENT A*		TREATMENT B*		TREATMENT C*	
	1**	2	1	2	1	2
I	13	76.9	169	62.1	160	16.3
II	9	22.2	94	7.4	82	6.1
III	13	7.7	107	19.6	112	12.5
IV	11	0.0	124	10.5	96	6.3

* Treatment A - Diet 1 throughout 8 weeks (1 hatch)
B - Diet 1 the first 4 weeks and diet 4 the second 4 weeks (summary of 7 hatches)
C - Diet 4 throughout 8 weeks (summary of 7 hatches)

** 1 - Total Chicks
2 - Percent dead

chicks on the other two treatments. However, progeny of dams fed Group III and IV diets showed a slightly higher mortality in treatment C than on treatment A, but lower than on treatment B. These results clearly indicate that maternal diet does bear a direct effect upon the mortality of progeny and that a complete broiler ration (diet 4) fed to the progeny will lower the mortality rate to a greater extent.

The effect of maternal diet upon the hatching weight of progeny is summarized in Table XI. It must be remembered that the hatching weights of these chicks represented only those chicks that survived at 8 weeks. In other words, the hatching weights of those chicks that died were not included in the data. The Group I diet appeared to produce heavier chicks than the 3 other diets. One reason for this may be attributed to larger egg size laid by dams fed Group I diet. Although no genetic measures were undertaken to determine the relation of diet to egg size, just why the phenomenon occurred may possibly be attributed to chance in the selection of pullets for larger egg size in one group and of pullets for smaller egg size in the other groups.

TABLE XI

EFFECT OF MATERNAL DIET UPON THE HATCHING WEIGHT OF PROGENY

MATERNAL DIET	MALES	FEMALES	MEAN OF MALES AND FEMALES
		(grams)	
I	40.7(112)*	41.1(86)	40.9(198)
II	39.0(84)	37.6(87)	38.3(171)
III	38.9(102)	38.3(94)	38.6(196)
IV	38.2(112)	37.1(94)	37.6(206)

* Number in parenthesis refers to number of chicks that survived at 8 weeks of age.

Tables XII, XIII, and XIV represent a summary of 4-week and 8-week growth rates of chicks on treatments A, B, and C, respectively. It must be borne in mind that the hatching weights included in these tables are in no way influenced by chick diet. They are inserted for the sake of comparison with 4-week and 8-week weights on the 3 treatments.

Irrespective of chick diet, Group I chicks grew at the slowest rate of all chicks at 4 weeks and 8 weeks of age. The only exception is on treatment A at 8 weeks of age. The average weight of the 3 males in Group I was little heavier than the mean weight of males and mean weight of females from Group IV dams. Had any female chicks been included in Group I, the mean weight of both sexes might have been lower than that of Group IV chicks. No apparent differences were noted in the mean weights of both sexes of Group II, III, and IV chicks at 4 and 8 weeks of age, as influenced by maternal diet.

A comparison of the 3 treatments show that, in most cases, all the progeny grew faster on treatment C than on treatments A or B. Group II and III chicks at 4 weeks of age and Group III chicks at 8 weeks of age appeared to grow faster on treatment A than on treatment B. These small differences probably would have disappeared if more chicks had been available on treatment A. For this reason, no comparison can be made in growth rates of chicks fed treatments A and the other 2 treatments because of the small number of survivors at 8 weeks.

At 4 weeks of age on treatment B, females from Group I, II, and III grew at a faster rate than the males of the same groups, respectively. Likewise, on treatment C, the growth rate of females from Group III and IV was greater than that of males from the same groups, respectively.

TABLE XII

EFFECT OF MATERNAL DIET UPON GROWTH RATES OF PROGENY AT 4 WEEKS AND 8 WEEKS OF AGE ON TREATMENT A

MATERNAL DIET	TREATMENT A		
	MALES	FEMALES	MEAN OF MIXED SEXES
	AVERAGE HATCHING WEIGHTS (GRAMS)		
I	40.0(3)*	--	40.0(3)
II	40.0(4)	36.7(3)	38.4(7)
III	35.8(5)	34.9(7)	35.4(12)
IV	37.4(8)	34.7(3)	36.1(11)
	AVERAGE 4-WEEK WEIGHTS (GRAMS)		
I	164.0	--	164.0
II	217.5	184.7	201.1
III	232.8	184.3	208.6
IV	185.9	165.3	175.6
	AVERAGE 8-WEEK WEIGHTS (GRAMS)		
I	617.0	--	617.0
II	649.5	598.6	624.1
III	729.6	601.3	665.5
IV	650.8	554.7	602.8

* Number in parenthesis refers to number of survivors at 8 weeks of age.

TABLE XIII

EFFECT OF MATERNAL DIET UPON GROWTH RATES OF PROGENY AT 4 WEEKS AND 8 WEEKS OF AGE ON TREATMENT B

MATERNAL DIET	TREATMENT B		
	MALES	FEMALES	MEAN OF MIXED SEXES
	AVERAGE HATCHING WEIGHTS (GRAMS)		
I	42.5(42)*	41.5(22)	42.0(64)
II	39.4(43)	38.7(44)	39.5(87)
III	39.3(45)	40.1(41)	39.7(86)
IV	39.5(57)	39.0(54)	39.3(111)
	AVERAGE 4-WEEKS WEIGHTS (GRAMS)		
I	175.7	194.5	185.1
II	195.8	199.0	197.4
III	183.8	204.3	194.1
IV	217.1	210.0	213.6
	AVERAGE 8-WEEK WEIGHTS (GRAMS)		
I	624.9	605.9	615.4
II	689.1	621.2	655.2
III	645.4	613.9	629.7
IV	717.5	630.1	673.8

* Number in parenthesis refers to number of survivors at 8 weeks of age.

TABLE XIV

EFFECT OF MATERNAL DIET UPON GROWTH RATES OF PROGENY AT 4 WEEKS AND 8 WEEKS OF AGE ON TREATMENT C

MATERNAL DIET	TREATMENT C		
	MALES	FEMALES	MEAN OF MIXED SEXES
	AVERAGE HATCHING WEIGHTS (GRAMS)		
I	39.7(67)*	40.6(64)	40.2(131)
II	37.7(37)	37.3(40)	37.5(77)
III	41.5(52)	40.0(46)	40.8(98)
IV	37.6(47)	37.6(37)	37.6(84)
	AVERAGE 4-WEEK WEIGHTS (GRAMS)		
I	234.5	214.1	224.3
II	260.2	236.5	248.4
III	239.2	243.5	241.4
IV	247.1	254.7	250.9
	AVERAGE 8-WEEK WEIGHTS (GRAMS)		
I	729.0	626.8	677.9
II	759.5	641.9	700.7
III	733.6	673.7	703.7
IV	711.1	675.9	693.5

* Number in parenthesis refers to number of survivors at 8 weeks of age.

However at 8 weeks of age on both diets, all the males were heavier than the females in the groups under study. No attempt was made to determine statistically the significance of a sex difference at 4 weeks of age on treatments B and C, because of the small number of progeny involved.

The results as summarized in Tables XII, XIII, and XIV show that the maternal diet does produce an effect in the growth rate of the progeny and that the growth rates are also influenced by chick diet, regardless of the source of progeny.

The effects of maternal diet upon feather pigmentation of progeny are summarized in Table XV. Irrespective of chick diet, the percentage of black and/or white feathers was greatest in Group I chicks. The addition of synthetic folic acid to the maternal diet reduced the percentage to a greater degree. The percentage of abnormal feather pigmentation of Group I chicks was much less on treatment C than on the other 2 treatments. The reason for the lowered percentage of BW feathers in Group IV chicks on treatment C than on treatment B may be explained on the basis that folic acid stores in the bodies of the chicks were not great enough to carry the chicks throughout the first 4 critical weeks of life on treatment B. These results strongly indicate the tremendous influence of maternal diet as well as chick diet upon abnormal feather pigmentation in progeny. In all instances, irrespective of chick diet, the percentage of abnormal feather pigmentation was greater in males than in females, thereby indicating a sex difference in this respect.

Feather development is also greatly influenced by maternal diet, as is shown in Table XVI. The percentage of chicks bearing normal feathers from the standpoint of structure and pigmentation was lowest in Group I

TABLE XV

EFFECT OF MATERNAL DIET UPON FEATHER PIGMENTATION OF PROGENY

MATERNAL DIET	TOTAL SURVIVORS AT 8 WEEKS		NO. BW FEATHERS*		PERCENT BW FEATHERS		MEAN PERCENT BW FEATHERS OF MIXED SEXES
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	
TREATMENT A							
I	3	0	3	0	100	0	100.0
II	4	3	1	0	25	0	14.3
III	5	7	0	0	0	0	0.0
IV	8	3	1	0	12.5	0	9.1
TREATMENT B							
I	42	22	31	12	73.7	54.5	67.1
II	43	44	3	1	6.9	2.2	4.6
III	45	41	5	2	11.1	4.9	8.1
IV	57	54	5	3	8.1	5.6	7.2
TREATMENT C							
I	67	67	37	22	55.2	32.8	44.1
II	37	40	4	0	10.8	0.0	5.2
III	52	46	9	3	17.3	6.5	12.2
IV	53	57	2	0	3.8	0.0	1.8

* BW represents black and/or white feathers.

TABLE XVI

EFFECT OF MATERNAL DIET UPON FEATHER DEVELOPMENT OF PROGENY

MATERNAL DIET	TOTAL SURVIVORS AT 8 WEEKS ♂♂	TOTAL SURVIVORS AT 8 WEEKS ♀♀	PERCENT NORMAL CHICKS ♂♂	PERCENT NORMAL CHICKS ♀♀	MEAN PERCENT MIXED NORMAL SEXES	FEATHER SCORE FOR NORMAL CHICKS ♂♂	FEATHER SCORE FOR NORMAL CHICKS ♀♀	MEAN FEATHER SCORE OF MIXED SEXES	PERCENT B_{10} ♂♂	PERCENT B_{10} ♀♀	MEAN PERCENT B_{10} OF MIXED SEXES
TREATMENT A											
I	3	0	0	0	0	0	0	0	0	0	0
II	4	3	75	100	85.7	100	100	100	0	0	0
III	5	7	80	100	91.7	100	100	100	20	0	8.3
IV	8	3	87.5	100	90.9	98.6	100	99.3	0	0	0
TREATMENT B											
I	42	22	21.4	36.4	26.6	98.4	98.8	98.6	40.4	22.7	34.4
II	43	44	90.7	95.5	93.1	98.9	99.8	99.4	6.9	2.2	4.6
III	45	41	73.3	92.7	82.6	97.2	98.9	98.1	22.2	2.4	12.8
IV	57	54	82.5	98.1	90.1	98.5	99.3	98.9	8.1	3.7	6.3
TREATMENT C											
I	67	67	32.8	61.2	47.0	97.9	98.7	98.3	32.8	14.9	23.9
II	37	40	89.2	100.0	94.8	99.3	99.7	99.5	0.0	0.0	0.0
III	52	46	80.8	89.1	84.7	99.4	99.9	99.6	7.7	6.5	7.1
IV	53	37	92.5	100.0	95.6	98.4	99.9	99.2	3.8	0.0	2.2

chicks, irrespective of chick diet. Chicks from the folic acid supplemented groups (Group II & III) compared favorably with those from Group IV on all 3 treatments from the standpoint of feather development. All the chicks bearing normal feathers had a very good feather rating, as indicated by the feather score column. The chicks from each group (except Group I) could not be distinguished from one another in regard to the degree of feather development and general appearance.

Those chicks bearing normal feather pigmentation but not normal feather development were found in greatest numbers from Group I dams. The addition of synthetic folic acid to the maternal diet reduced the "B_{10} deficiency" greatly, as compared with Group IV chicks. The percentage of B_{10} deficiency was lowest on treatment C for all the progeny.

In all instances, regardless of chick diet, males showed a greater percentage of abnormal feather development than females of the same groups, thereby indicating a sex difference in regard to the degree of normal feather development.

To illustrate more clearly the general appearance of chicks bearing abnormal feather pigmentation and/or poor feather development, figures 1 to 6 are enclosed in the following pages: figure 1 represents a normal chick at 8 weeks of age from the standpoint of feather pigmentation and development. The chick in figure 2 is one of the progeny from Group I dams fed the folic acid-low basal diet and represents a typical example of abnormal feather pigmentation. Notice the large black and white areas in the primary and secondary wing feathers. Also notice the varying shades of red pigment on the wing coverts. Figure 3 represents a typical chick with a B_{10} deficiency which is characterized by broken wing feather tips and by frayed feathers on the dorsal region of the chick. Although a few chicks were found to be abnormal in both feather pigmentation and development, most chicks bearing a B_{10} deficiency showed normal feather pigmentation. Another type of B_{10} deficiency is found in figure 4, in which the wing feather tips are not broken off but are somewhat curled and rough appearing. In addition, the feathers growing on the dorsal area of the chick are very fuzzy, down-like, and very light in color. A sharp contrast is noticed between this chick and a chick shown in figure 5, which is normal in every respect. Notice the fine looking, well-formed feathers everywhere. Figure 6 shows the 2 chicks in figures 4 and 5 together to give an illustration on the effect of maternal diet upon feather pigmentation and structure in progeny.

4. Chick Experiment 2. The high percentage of abnormal feather pigmentation obtained with Group I progeny in Chick Experiment 1 led to the set-up of this experiment to determine whether or not a dietary factor added to the basal diet would reduce the percentage of abnormal feather pigmentation to a minimum.

Biginning with the 16th hatch, all the Group I progeny were wing-pedigreed and all the other progeny from the 3 other groups of dams were discarded at hatching time. An effort was made to segregate the chicks in such a way that the progeny from each dam would be represented in equal numbers on both the basal and supplemented diets. After the first 4 weeks on their respective diets, the survivors were then fed diet 4 for another 4-week period.

Table XVII represents a summary of the mortality and growth data of progeny from Group I dams. In most instances, the mortality was greater

Figure 1. A NORMAL COCKEREL AT 8 WEEKS OF AGE

Figure 2. A COCKEREL FROM ONE OF GROUP I DAMS, SHOWING TYPICAL ABNORMAL FEATHER PIGMENTATION

Figure 3. A TYPICAL CHICK FROM THE PROGENY OF GROUP I DAMS, SHOWING TYPICAL POOR FEATHER STRUCTURE

Figure 4. ANOTHER EXAMPLE OF POOR FEATHER STRUCTURE, IN WHICH THE FEATHERS ARE CURLED AND FRAYED

Figure 5. A NORMAL PULLET FROM SAME HATCH AS THE CHICK IN FIGURE 4

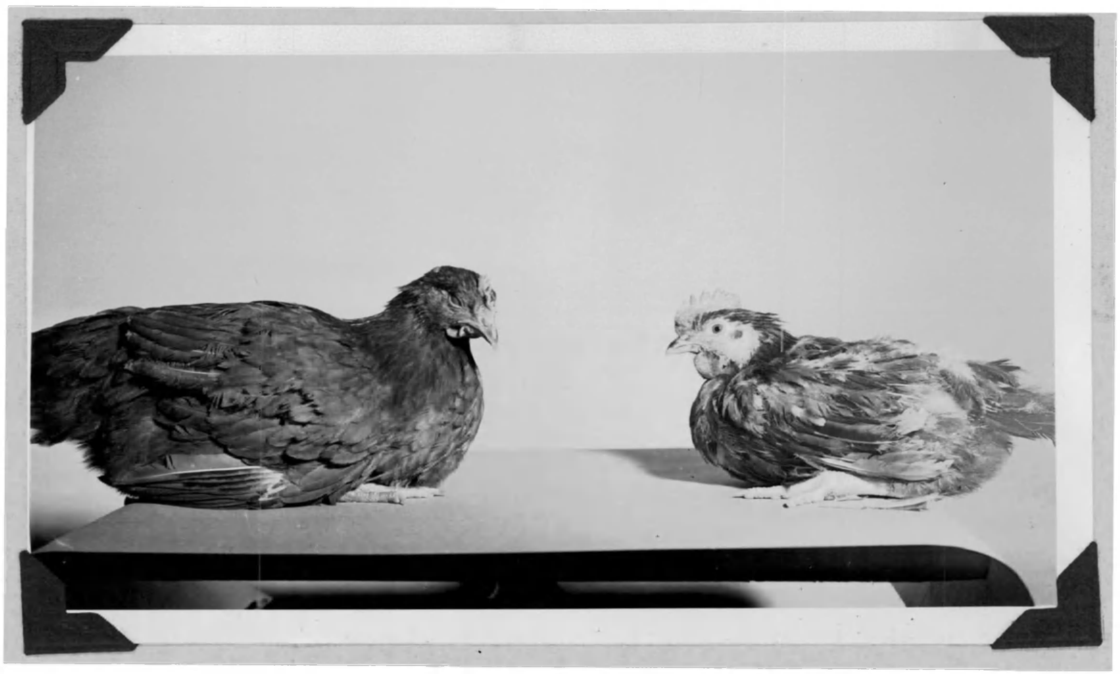


Figure 6. COMPARISON OF CHICKS IN FIGURES 4 AND 5

TABLE XVII

EFFECT OF CHICK DIET UPON MORTALITY AND GROWTH RATES OF PROGENY FROM DAMS FED DIET 1

DATE OF HATCH	DIET	NO. CHICKS AT START	NO. CHICKS AT 8 WEEKS	MEAN BODY WEIGHTS OF MIXED SEXES AT 4 WEEKS (grams)	MEAN BODY WEIGHTS OF MIXED SEXES AT 8 WEEKS (grams)
3/26/48	Basal	20	10	174.1	560.7
	Feces #1 ad libitum*	19	3	191.3	617.0
4/9	Basal	20	9	200.1	602.6
	5% feces #1*	19	11	185.7	532.9
4/20	Basal	18	14	212.7	610.4
	5% feces #2*	13	7	191.5	614.9
5/4	Basal	19	6	153.0	485.6
	1% lysine	15	8	199.9	596.5
6/1	Basal	20	11	206.6	568.4
	200 mcg. folic acid/100 gms.	20	17	249.6	657.1
6/11	Basal	17	9	223.1	582.1
	5% dried cow manure	17	11	199.4	547.2
6/25	Basal	16	7	249.6	708.2
	5% feces #2*	18	8	232.6	711.0
7/6	Basal	15	10	235.5	548.6
	6.6 mg. Parvo/100 gms.	15	15	261.5	604.6
7/20	Basal	16	13	240.5	661.2
	1% lysine + 200 mcg. folic acid/100 gms.	14	12	271.6	718.4
8/23	Basal	11	10	228.0	669.1
	2 egg yolks/1 kilogram**	9	8	255.7	682.1

* Feces #1 - feces obtained from Group IV dams
Feces #2 - feces obtained from Group I dams

** Egg yolks were obtained from eggs laid by Group I dams on the basal diet than on the supplemented diet. However, the last 3 hatches had the least mortality on the basal diet of all the hatches combined.

When chicks were fed fresh hen feces ad libitum or at a level of 5 percent, the mortality was somewhat higher than on the other supplemented diets. Hen feces fed at 5 percent, regardless of source, depressed growth over the basal group. However, the feces fed ad libitum did increase the growth rate over the basal group in spite of the high mortality incurred. The reason for using fresh feces was because folic acid activity is destroyed by heating (Cheldelin et al., 1943).

At 4 weeks of age, of all the supplements that produced a growth response as compared with the basal diet, the greatest growth response was produced by lysine, folic acid, and a combination of lysine and folic acid in the following order. Dried cow manure at the level of 5 percent depressed the growth rate.

It will be interesting to note that the basal group chicks grew at a heavier rate as the hatches extended into the summer months. In all hatches except hatches 4/9 and 6/11 the supplemented groups were heavier than the basal groups at 8 weeks of age. In hatch 6/11 the depressing effects of cow manure may have been so great during the first 4 weeks that normal growth was not fully resumed during the second 4 weeks on a normal broiler diet.

The effects of the supplemented diets upon feather pigmentation are summarized in Table XVIII. The mean percentage of BW feathers of mixed sexes shows that all the supplements added to the basal diet (except hatch 4/9) did lower the degree of abnormal feather pigmentation to a

TABLE XVIII

EFFECT OF CHICK DIET UPON FEATHER PIGMENTATION
OF PROGENY FROM DAMS FED DIET 1

RATE OF HATCH	CHICK DIET*	TOTAL SURVIVORS AT 8 WEEKS		PERCENT BW		MEAN PERCENT BW OF MIXED SEXES
		MALES	FEMALES	MALES	FEMALES	
3/26	Basal	5	5	60	100	80
	Feces #1 ad libitum	0	3	0	33.3	33.3
4/9	Basal	4	5	25	100	66.6
	5% feces #1	8	3	87.5	66.6	81.8
4/20	Basal	9	5	77.7	80	78.5
	5% feces #2	3	4	66.6	25	42.8
5/4	Basal	4	2	75	100	83.3
	1% lysine	3	5	66.6	20	37.5
6/1	Basal	6	5	100	80	90.9
	200 mcg. folic acid/100gms.	11	6	18.1	0	11.7
6/11	Basal	4	5	75	60	66.6
	5% dried cow manure	7	4	71.4	25	54.5
6/25	Basal	5	2	60	50	57.1
	5% feces #2	2	6	0	33.3	25
7/6	Basal	6	4	100	50	80
	6.6 mg. Parvo/100 gms.	4	11	75	9.1	26.6
7/20	Basal	6	7	83.3	57.1	69.2
	1% lysine and 200 mcg. folic acid/100 gms.	9	3	33.3	0	25
8/23	Basal	4	6	100	50	70
	2 egg yolks/kilogram	4	4	50	50	50

* Diets are described in Table XVII

lesser or greater degree, as compared with the basal diet in each hatch. Folic acid and Parvo were the most effective supplements in reducing the degree of abnormal feather pigmentation to a minimum. Dried cow manure was the least effective of all supplements in this respect. In the feces experiments, 5 percent of feces #2 and feces #1 ad libitum reduced the abnormality to a small degree. However, 5 percent of feces #1 actually increased the abnormality over the basal group. Why the same source of feces fed ad libitum and at 5 percent would cause different responses cannot be explained. No conclusions can be drawn to the effectiveness of source and level of feces in the prevention of abnormal feather pigmentation because only one test was run with feces #1.

In the first 4 hatches, the percentage of abnormal feather pigmentation was greater in the females than in the males on the basal diet. The reverse was true for the next 6 hatches. With the exception of hatches 3/26 and 6/25, the males of all hatches showed greater percentage of abnormal feather pigmentation than the females on the supplemented diets.

In regard to the effect of supplemented diets upon feather development as summarized in Table XIX, lysine, folic acid, Parvo, combination of lysine and folic acid, egg yolks, dired cow manure, and one test of 5 percent of feces #2, all reduced vitamin B_{10} deficiency to a lower degree over the basal diet. The percentage of mixed sexes with normal feathers was greatest with folic acid, combination of folic acid and lysine, 1 trial of 5 percent hen feces #2, and Parvo. Dried cow manure and 5 percent of feces #1 were least effective from the standpoint of producing chicks with normal feathering.

TABLE XIX

EFFECT OF CHICK DIET UPON FEATHER STRUCTURE
OF PROGENY FROM DAMS FED DIET 1

DATE OF HATCH	CHICK DIET*	TOTAL SURVIVORS AT 8 WEEKS		PERCENT B_{10}		MEAN PERCENT B_{10} OF MIXED SEXES	PERCENT NORMAL CHICKS		MEAN PERCENT NORMAL MIXED SEXES
		MALES	FEMALES	MALES	FEMALES		MALES	FEMALES	
3/26	Basal	5	6	60	0	30	20	0	10
	Feces #1 _ad libitum_	0	3	0	0	0	0	66.6	66.6
4/9	Basal	4	5	75	20	44.4	25	0	11.1
	5% feces #1	8	3	37.5	66.6	45.4	0	33.3	9.1
4/20	Basal	9	5	11.1	20	14.3	22.2	20	21.4
	5% feces #2	3	4	33.3	0	14.3	33.3	75	44.4
5/4	Basal	4	2	25	100	50	25	0	16.6
	1% lysine	3	5	33.3	20	25	33.3	80	62.5
6/1	Basal	6	5	33.3	20	27.2	0	20	9.1
	200 mcg. folic acid/100 gms.	11	6	9.1	0	5.8	72.7	100	82.3
6/11	Basal	4	5	75	20	44.4	0	20	22.2
	5% dried cow manure	7	4	28.5	25	27.2	14.2	75	36.3
6/25	Basal	5	2	20	0	14.2	20	50	33.3
	5% feces #2	2	6	0	0	0	100	66.6	75.0

TABLE XIX (CONTINUED)

EFFECT OF CHICK DIET UPON FEATHER STRUCTURE
OF PROGENY FROM DAMS FED DIET 1

DATE OF HATCH	CHICK DIET*	TOTAL SURVIVORS AT 8 WEEKS		PERCENT B_{10}		MEAN PERCENT B_{10} OF MIXED SEXES	PERCENT NORMAL CHICKS		MEAN PERCENT NORMAL MIXED SEXES
		MALES	FEMALES	MALES	FEMALES		MALES	FEMALES	
7/6	Basal	6	4	16.6	25	20.0	0	50	20.0
	6.6 mg. Parvo/100 gms.	4	11	0	0	0	25	90.9	73.3
7/20	Basal	6	7	33.3	57.1	46.1	16.6	28.5	23.1
	1% lysine and 200 mcg. folic acid/100 gms.	9	3	0	0	0	66.6	100	75.0
8/23	Basal	4	6	0	50	30	0	33.3	20.0
	2 egg yolks/kilogram	4	4	25	0	12.5	50	50	50.0

* Diets are described in Table XVII

Because of the small number of survivors at 8 weeks of age, no attempt was made to determine statistacally a sex significance of abnormal feather pigmentation and of vitamin B_{10} deficiency.

5. Chick Experiment 3. This experiment was devoted to chicks obtained from mass matings on the College Poultry Farm. To observe the effect of the first 2-weeks' diet upon subsequent growth, with folic acid as the limiting factor, three series of experiments were conducted for a period of 6 weeks. In series A the day-old chicks were weighed individually and only those weighing 40-44 grams were selected and given their respective diets at hatching time. The selection of chicks in both series B and C was slightly different from that in series A. All the day-old chicks were fed the hen basal diet 1 at hatching time in series B and diet 4, typical of broiler mashes, in series C. At 2 weeks of age all the survivors were weighed individually and normally distributed with a range of 55 to 140 grams in series B and with a range of 60 to 140 grams in series C. The mean weight was 95 and 100 grams in series B and series C, respectively. Only those chicks within a range of 90-110 grams in both series were selected for the experiments, and all the other chicks were discarded. The selected chicks were then fed their respective diets from the second to the sixth week. Repitition of 3 experiments in each series was made, with the exception of the 300 microgram level. Only one test was run at this level.

The effects of the first 2-weeks' diet upon subsequent growth rates, feather pigmentation and feather development of chicks fed supplements from the second to sixth week are summarized in Table XX. In series A in which the chicks were fed their respective diets from hatching time on, those chicks fed diet 4 produced the greatest growth response, as indicated by

TABLE XX

EFFECT OF FIRST 2 WEEKS DIET UPON SUBSEQUENT GROWTH RATES, FEATHER PIGMENTATION AND FEATHER DEVELOPMENT OF CHICKS FED SUPPLEMENTS FROM THE SECOND TO SIXTH WEEK

CHICK DIET 2nd TO 6th WEEK	NO. CHICKS AT 2 WEEKS	AVERAGE GAIN IN 4 WEEKS	PERCENT GAIN OVER BASAL	FEED EFFICIENCY*	BW	B_{10}
SERIES A - CHICKS ON SUPPLEMENTS AT HATCHING TIME						
		grams				
Basal (diet 1)	49	305.2	--	0.322	4	3
200 mcg. folic acid/100 gms.	49	339.2	111.1	0.406	0	0
400 mcg. folic acid/100 gms.	30	352.2	115.4	0.447	0	0
Broiler mash (diet 4)	50	394.8	129.4	0.411	0	1
SERIES B - CHICKS ON DIET 1 FIRST 2 WEEKS						
Diet 1	30	303.7	--	0.361	2	2
50 mcg. folic acid/100 gms.	30	321.1	105.7	0.334	3	4
100 mcg. folic acid/100 gms.	30	327.1	107.7	0.346	1	3
200 mcg. folic acid/100 gms.	30	321.5	105.9	0.327	2	4
300 mcg. folic acid/100 gms.	10	315.6	103.9	0.338	2	0
400 mcg. folic acid/100 gms.	20	318.3	104.8	0.349	2	3
Diet 4	30	351.6	115.8	0.345	3	2
SERIES C - CHICKS ON DIET 4 FIRST 2 WEEKS						
Diet 1	29	315.5	--	0.333	1	3
50 mcg. folic acid/100 gms.	29	344.2	109.1	0.352	2	4
100 mcg. folic acid/100 gms.	29	359.9	114.1	0.346	4	2
200 mcg. folic acid/100 gms.	29	316.3	100.3	0.323	1	1
300 mcg. folic acid/100 gms.	9	302.4	95.8	0.333	0	0
400 mcg. folic acid/100 gms.	29	345.0	109.4	0.352	3	7
Diet 4	29	344.2	109.1	0.356	0	0

* Feed Efficiency= $\frac{\text{Total gain in weight}}{\text{Total feed consumption}}$

the percent gain over the basal chicks. The chicks fed the basal diet grew the slowest. Of the 8 chicks that showed either abnormal feather pigmentation or poor feather structure, 7 were from the group fed the basal diet.

In series B when day-old chicks were fed diet 1 for 2 weeks, followed by folic acid supplements from the second to the sixth week, the growth response was less than for the corresponding groups in series A. The groups fed the basal diet and diet 4 produced the slowest and the most rapid growth response, respectively, in series B. The degree of abnormal feather pigmentation and feather structure was equally distributed among all groups in the series, even on diet 4.

When day-old chicks were fed diet 4 during the first 2 weeks of life and then transferred to folic acid supplements at 2 weeks of age in series C, the group fed the basal diet grew at a slower rate than all the folic acid-supplemented groups except the 300 microgram level. In this case, no comparison between the 300 microgram level and the other groups in the series should be considered because of the small number of chicks involved in the 300 microgram group. The growth response on the 50, 100, and 400 microgram levels was equivalent to that obtained in the group fed diet 4.

A comparison of series A, B, and C shows that the group fed the basal diet in series C grew at a slightly heavier rate than the corresponding group in series A and B, a fact indicating a critical need for folic acid in the basal diet at hatching time (diet 4 contained 159.5 micrograms of folic acid per 100 grams of diet).

From the standpoint of feather pigmentation and structure, the degree of abnormal feather pigmentation and poor feather structure was lowest in series A, and equally distributed in series B and C.

Feed efficiency figures for series A, B, and C are submitted in Table XX. Mortality was negligible in the 3 series.

A fourth series, series D, was conducted to evaluate the nutritional properties of Parvo, a crude folic acid supplement, in a practical ration low in folic acid. The procedure for selecting chicks and placing them on their respective diets was similar to that explained in series A. Twenty chicks were used per group; only one experiment was conducted. After being on their respective diets during the first 4 weeks, the survivors were fed diet 4 during the second 4 weeks. Feed consumption records were kept during the first 4 weeks.

The results of series D are summarized in Table XXI. The addition of Parvo to a folic acid-low basal ration appeared to produce a growth response equivalent to that on diet 4 at 4 weeks of age. Several levels of Parvo did not produce a growth rate greater than that obtained on the basal diet. The 5,100 microgram level produced a greater growth response than the broiler ration (diet 4).

At 8 weeks of age the mean body weight of mixed sexes indicated that Parvo fed at a minimum level of 3,400 micrograms per 100 grams of diet to supply 100 micrograms of folic acid per 100 grams of diet supported normal growth in chicks fed a folic acid-low ration during the first 4 weeks, followed by diet 4 the second 4 weeks, as compared with chicks fed diet 4 throughout 8 weeks.

Chicks showing abnormal feather pigmentation were found in greatest numbers on the basal diet, and next greatest on the lowest level of Parvo. It will be interesting to note that of all the 15 chicks showing black and/or white feathers, 13 were males.

TABLE XXI

EFFECT OF PARVO SUPPLEMENTS IN A PRACTICAL DIET LOW IN FOLIC ACID WHEN FED TO NEW HAMPSHIRE DAY-OLD CHICKS

LEVEL OF PARVO ADDED TO BASAL	MEAN BODY WEIGHTS OF MIXED SEXES 4 WEEKS	8 WEEKS	FEED EFFICIENCY 4 WEEKS**	BW		B_{10}
	(grams)			♂♂	♀♀	(all males)
None	231.4 (20)*	690.4	0.418	5	0	1
340 mcg./100 gms.	237.0 (20)	684.9	0.431	3	0	0
850 mcg./100 gms.	231.7 (19)	720.4	0.407	1	1	1
1750 mcg./100 gms.	242.8 (18)	718.9	0.433	1	0	0
3400 mcg./100 gms.	237.6 (20)	737.8	0.403	0	0	0
5100 mcg./100 gms.	258.2 (19)	737.9	0.432	0	0	0
6800 mcg./100 gms.	241.5 (19)	728.2	0.404	2	0	0
10200 mcg./100 gms.	246.4 (20)	737.1	0.428	0	0	1
13600 mcg./100 gms.	232.9 (18)	744.7	0.404	1	1	1
Diet 4	246.1 (19)	739.1	0.392	0	0	0

* Number in parenthesis refers to number of survivors at 8 weeks of age

** Feed Efficiency= $\frac{\text{Total gain in weight}}{\text{Total feed consumption}}$

6. Summary of chick experiments. In Chick Experiment 1, chicks from Group I dams showed a greater mortality rate, a slower growth rate, and a greater percentage of abnormal feather pigmentation and of poor feather structure than all other progeny, irrespective of chick diet. Group I progeny did better in all respects on a normal broiler ration than on the basal diet. Males appeared to be more sensitive than females from the standpoint of abnormal feather pigmentation and feather structure.

Feather pigmentation studies with Group I progeny in Chick Experiment 2 showed that folic acid and Parvo were the most effective supplements in reducing black and/or white feathers to a minimum on a basal diet low in folic acid. Dried cow manure was less effective in this respect. A combination of folic acid and lysine; Parvo; and folic acid reduced abnormal feather structure to a lesser degree.

A comparison of the first-2 weeks' diet upon subsequent growth in chicks obtained from farm mass matings showed some differences, as indicated in Chick Experiment 3. The growth response for the folic acid supplemented diets was greatest in series A. Those chicks fed the basal diet in the 3 series were the heaviest in series C. The degree of abnormal feather pigmentation and feather structure was greater in series B and C than in series A.

A sample of Parvo in series D showed to be a satisfactory supplement for chicks fed a folic acid-low basal ration. A level of 3,400 micrograms per 100 grams of basal diet during the first 4 weeks of life, followed by a normal broiler ration the second 4 weeks produced a growth response equivalent to that obtained with chicks fed a normal broiler ration throughout 8 weeks.

DISCUSSION

A. Studies with Laying Pullets

The folic acid requirement for normal egg production in New Hampshire pullets fed a practical diet low in folic acid is somewhere around 33.8 micrograms per 100 grams of diet, according to the data included in this manuscript. This is in general agreement with that of Schweigert et al. (1948) who reported that normal egg production was maintained on a folic acid-low ration containing 42 micrograms of folic acid per 100 grams of diet.

Since a practical ration containing 26.3 micrograms of folic acid per 100 grams of diet required additional folic acid for normal egg production, this finding is not in agreement with that of Taylor (1947) who reported normal egg production on a semi-synthetic ration containing 12 micrograms of folic acid per 100 grams of diet. This discrepancy may possibly be explained on the basis of breed, age, diet, and environment. Taylor (1947) used yearling White Leghorn hens fed a semi-synthetic diet, whereas New Hampshire pullets fed a practical diet were employed in this manuscript.

In hatchability studies, a practical ration containing 33.8 micrograms of folic acid per 100 grams of diet was adequate to maintain normal hatchability in New Hampshire pullets. When the folic acid activity of this ration was lowered to 26.3 micrograms per 100 grams of diet, folic acid supplementation was required for normal hatchability. These findings are confirmed both by Taylor (1947) who reported that a semi-synthetic ration containing 12 micrograms of folic acid per 100 grams of diet was not adequate to maintain normal hatchability and by Schweigert et al. (1948) who found that a practical ration containing 42 micrograms of folic acid per 100 grams of diet was adequate to support normal hatchability.

Even though a practical ration containing only 33.8 micrograms of folic acid per 100 grams of ration is adequate for normal egg production, fertility, and hatchability, this ration did lower the folic acid activity in eggs laid by dams fed the basal diet than in those laid by dams fed the folic acid supplemented diets. This finding is in general agreement with that of Schweigert et al. (1948).

No differences in the general appearance of progeny were observed in basal diet 1 described in this manuscript. The results obtained in this manuscript on diet 1 revealed differences in hemoglobin levels in favor of the folic acid supplemented diets. Thus these results would indicate that a practical ration containing 33.8 micrograms of folic acid per 100 grams of diet would need additional folic acid to maintain hemoglobin levels, since Schweigert et al. (1948) reported no differences in hemoglobin levels of laying pullets fed a ration containing 42 micrograms of folic acid per 100 grams of diet.

B. Studies with chicks

1. Chick Experiment 1. No mention was made of any effect of maternal diet upon mortality, growth, and feathering in chicks in the work of Taylor (1947) and of Schweigert et al. (1948). In growth studies with turkey poults secured from dams fed the basal diet, Schweigert et al. (1948) did not report any effects of maternal diet upon feather pigmentation and feather structure. Thus no comparison could be made between the 3 basal diets in this respect.

The results obtained in Chick Experiment 1 in this manuscript clearly indicate the influence of maternal diet upon the growth response of progeny. These results are substantiated by the work of Rubin and Bird (1947) who suggested that the unknown growth factor was transmitted by the hen to her progeny through the egg, and by Robblee, Nichol, Cravens, Elvehjem, and Halpin (1948) who stated that the growth response of the chick is influenced by the dam's diet.

That the males showed a greater incidence of abnormal feather pigmentation and feather structure than females suggest the possibility of a sex difference in the folic acid requirement for normal feathering.

2. Chick Experiment 2. Lillie and Briggs (1947 c) reported a level of 150 micrograms of folic acid per 100 grams of purified diet to be adequate for normal feather pigmentation in New Hampshire chicks obtained from dams fed a normal breeder mash. The fact that the addition of 200 micrograms of synthetic folic acid per 100 grams of a folic acid-low practical diet did not prevent entirely the occurrence of abnormal feather pigmentation in progeny from dams fed a folic acid-low ration suggests the possibility of a greater folic acid requirement for progeny from dams fed a folic acid-low ration than for progeny from dams fed a normal ration, from the standpoint of feather pigmentation. This hypothesis is strongly supported by the work of Luckey, Moore, Elvehjem and Hart (1947) in that dietary differences in the diet of hens used as a source of chicks could affect the bodily reserves of chicks which would, in turn, affect the chick growth responses on similar diets in experiments conducted at different times.

The increased growth response on basal diet 1 due to lysine supplementation may possibly be attributed to a slight lysine deficiency in the basal diet. According to the lysine activity of feedstuffs evaluated by Almquist (1946), basal diet 1 contained approximately 0.77 percent of lysine, and the lysine requirement for chicks is approximately 0.9 percent (Grau, Kratzer, and Asmundson, 1946).

The growth-depressing effect of dried cow manure may possibly be explained on the basis of a growth-inhibiting factor present in diet 1. Csonka and Olsen (1949) presented some very interesting facts about apparent antagonism between dried cow manure and casein. One lot of hens was fed a soybean meal diet and the other lot, a 20 percent casein diet. The progeny from these 2 lots were fed a normal broiler ration containing 5 percent of dried cow manure. The growth response of progeny from casein-fed hens was greatly depressed, as compared with that of progeny from soybean meal-fed hens. The authors concluded that a growth-inhibiting factor in casein was transmitted by the hen to her progeny through the egg which depressed the growth-promoting properties of dried cow manure in a normal broiler ration. In this respect, the growth-inhibiting factor under study may possibly be closely related to that present in dried skim milk in diet 1, which might account for the growth depression of folic acid-deficient chicks on basal diet 1. The growth-inhibiting factor could not possibly be present in the other ingredients, including fish meal, in diet 1 because no growth depression was observed on a diet containing 4 and 5 percent of fish meal and of dried cow manure, respectively, when fed to Rhode Island Red chicks, according to the work of Lillie, Marsden, Groschke and Bird (1949).

In growth and feather pigmentation studies with Group I progeny, bacterial synthesis should be given some consideration in the feces experiments.

Teply, Krehl and Elvehjem (1947) reported that excess niacin in a purified diet fed to rats increased the synthesis of folic acid and that excess folic acid increased the synthesis of niacin. Wright and Welch (1944) showed that deficient rats excreted more folic acid in feces than normal rats on a purified diet containing no succinylsulfathiazole. The diet of deficient rats contained milk. Richter and Rice (1945) reported that rats fed a ration deficient in all components of the vitamin B complex thrived on feces secured from normal rats. Mortality was lowered, and normal growth responses resulted from feeding normal rat feces to deficient rats.

Evidence was presented by McGinnis, Stevens and Groves (1947) to show that synthesis of an unidentified chick growth factor in hen feces took place after excretion rather than to any extent in the digestive tract. Incubation at 72 hours at 30 degrees Centigrade stimulated synthesis of the unknown growth factor in hen feces, whereas feces in the frozen state contained little or none of the factor. The feces were collected from White Leghorn hens fed a deficient diet.

In the light of these findings, it was hoped that the addition of fresh hen feces to a chick basal diet low in folic acid activity would throw further light on the role of bacterial synthesis, from the standpoint of growth and feathering of progeny secured from dams fed a folic acid-low practical ration. However, the growth-depressing effect of fresh hen feces, regardless of source of dietary level in chick diet, may be attributed to the presence of the urine constituents of fresh hen feces, as indicated by the work of Rubin, Bird and Rothchild (1946).

Furthermore, another possibility indicates that the folic acid-deficient chicks were not completely depleted of the unknown growth factor found in fresh hen feces and in dried cow manure (Rubin and Bird, 1946) because the chick basal diet (as well as the maternal diet) included fish meal which contained appreciable quantities of the unknown growth factor. Since the rats of Richter and Rice (1945) were deficient in all components of the vitamin B complex, they were more depleted than the folic acid-deficient chicks and, consequently, responded more readily to the unknown growth factor as well as other known vitamins in fresh feces from rats fed a normal diet.

From the standpoint of feather pigmentation, fresh hen feces collected from Group I and Group IV dams appeared to contain some "folic acid" activity. Just why 5 percent of feces from Group IV dams would produce a greater degree of abnormal feather pigmentation than the same source of feces fed ad libitum may be explained on the basis that the surviving chicks consumed more feces per unit of body weight than those fed 5 percent of feces. As a result, greater quantities of "folic acid" were consumed. However, no explanation is offered for the greater degree of abnormal feather pigmentation in the chicks fed 5 percent of feces from Group IV dams fed a normal laying mash than in those chicks of the same hatch on the basal diet.

3. Chick Experiment 3. The fact that the growth response of chicks fed 200 and 400 micrograms of folic acid per 100 grams of diet was greater in series A than in series C might possibly indicate a possibility of a greater folic acid requirement in the normal broiler ration from hatching time on. In this respect, maximum growth might be expected if chicks are to be fed 200 and 400 micrograms of folic acid per 100 grams of a folic acid-low ration from 2 weeks of age on. Furthermore, the addition of synthetic folic acid in a normal broiler ration at hatching time may possibly

prevent, or reduce to a minimum, abnormal feather pigmentation and poor feather structure if chicks are to be fed a folic acid-low ration from 2 weeks of age on.

The greater incidence of abnormal feather pigmentation in series B than in series A may be explained on the basis of the first 2 weeks diet in that the basal diet was so low in folic acid activity that the folic acid supplements from the second to the sixth week were not adequate to compensate for the deficiency incurred during the first 2 weeks of life. On the other hand, the experimental procedure for placing day-old chicks on diet 4 during the first 2 weeks, followed by folic acid supplements from the second to the sixth week (series C) did not lower materially the degree of abnormal feather pigmentation and feather structure, as had been anticipated. It was felt that diet 4 fed during the first 2 weeks of life would furnish adequate quantities of folic acid to lower the percentage of abnormal feather pigmentation in chicks, as compared with the corresponding groups in series B. However, this was shown not to be true. Chicks fed diet 4 in both series A and C did not show any evidence of black and/or white feather, but those fed the same diet in series B did because of the first 2 weeks of basal diet. Since diet 4 contained 159.5 micrograms of folic acid per 100 grams of diet, the possibility exists that diet 4, as well as diet 1, should contain at least 200 micrograms of folic acid per 100 grams of diet for the first 2 weeks of life, if abnormal feather pigmentation is to be prevented in chicks fed a folic acid-low basal ration containing folic acid supplements from 2 weeks of age on.

Since Parvo, a crude folic acid supplement, was an effective supplement in a folic acid-low ration, the results suggest that Parvo be incorporated in broiler mashes whenever the folic acid activity is sub-optimal due to omission of feedstuffs high in their folic acid activity or due to inclusion of feedstuffs in which their folic acid content was lowered for one reason or another. Depending upon the folic acid activity of Parvo, the incorporation of Parvo should be so as to supply a minimum of 100 micrograms of folic acid per 100 grams of diet for optimum growth and normal feathering.

CONCLUSIONS

1. A folic acid-low practical ration containing wheat middlings and also containing 33.8 micrograms of folic acid per 100 grams of diet was adequate for normal egg production, fertility, and hatchability of New Hampshire pullets.

2. When ground wheat was substituted for wheat middlings in a folic acid-low practical ration, the folic acid activity was lowered to 26.3 micrograms per 100 grams of diet. Additional folic acid was required for normal egg production, hatchability, and body weight maintenance. Fertility was not affected.

3. In a folic acid-low practical ration containing 33.8 micrograms of folic acid per 100 grams, the addition of folic acid improved body weight maintenance, increased hemoglobin values, and raised the folic acid activity of egg yolks of New Hampshire pullets.

4. No apparent differences were noted in down color, size, weight, and viability of progeny at hatching time as a result of maternal diet.

5. In viability studies, the progeny from dams fed a folic acid-low practical diet showed the greatest mortality of all progeny, irrespective of chick diet. No apparent difference were noted in the mortality of progeny from dams fed a folic acid-low ration supplemented with folic acid and from dams fed a typical laying mash.

6. In growth studies, progeny from dams fed the folic acid-low basal diet showed the slowest growth rate of all progeny, irrespective of chick diet. Generally speaking, growth of progeny from dams fed folic acid supplements was nearly equivalent to that of progeny from dams fed a typical laying mash.

7. Feather pigmentation studies showed that the degree of abnormal feather pigmentation was greatest in progeny from dams fed a folic acid-low basal diet and lowest in progeny from dams fed a typical laying mash, irrespective of chick diet.

8. A greater incidence of poor feather structure was found in progeny from dams fed the folic acid-low basal diet than in all other progeny, irrespective of chick diet.

9. Males appeared to be more sensitive than females to a folic acid deficiency from the standpoint of feather pigmentation and feather structure, thereby indicating a sex difference.

10. Of the 3 chick diets under study; namely, a folic acid-low basal ration throughout 8 weeks; this basal ration during the first 4 weeks, followed by a normal broiler mash during the second 4 weeks; and the normal broiler mash throughout 8 weeks, the progeny from dams fed the folic acid-low basal ration did better on the broiler mash throughout 8 weeks than on the other 2 chick diets from the standpoint of mortality, growth, feather pigmentation, and feather structure.

11. Synthetic folic acid and Parvo, a crude folic acid supplement, were the most effective supplements in reducing the degree of abnormal feather pigmentation to a minimum.

12. Dried cow manure and fresh hen feces depressed the growth of progeny from dams fed a folic acid-low basal diet. Some folic acid activity was present in these 2 compounds from the standpoint of abnormal feather pigmentation.

13. Farm mass-mated chicks fed a folic acid-low basal ration plus folic acid supplements from hatching time on for a period of 6 weeks

produced a greater growth response than those chicks fed either the folic acid-low basal diet or a typical broiler mash the first 2 weeks, followed by folic acid supplements from the second to the sixth week.

14. The degree of abnormal feather pigmentation was greater in farm mass-mated chicks fed either a folic acid-low basal ration or a typical broiler mash during the first 2 weeks of life than in those fed folic acid supplements from hatching time on.

15. For farm mass-mated chicks fed a folic acid-low basal ration during the first 2 weeks of life, the supplementation of synthetic folic acid in this basal ration from the second to the sixth week did not prevent abnormal feather pigmentation. This finding was true for those chicks fed a typical broiler mash during the first 2 weeks of life, followed by a folic acid-low basal diet plus folic acid supplements from the second to the sixth week.

16. For broiler mashes low in folic acid activity, the incorporation of Parvo to supply a minimum level of 100 micrograms of folic acid per 100 grams of diet supported normal growth and normal feather pigmentation in New Hampshire chicks during the first 4 weeks of life.

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APPENDIX

TABLE III

EFFECT OF DIET UPON FERTILITY AND HATCHABILITY OF NEW HAMPSHIRE PULLETS IN EXPERIMENT 1

DIET	NO. EGGS SET	NO. FERTILE EGGS	DEAD GERMS*			PIPPED EGGS	NO. CHICKS
			1	2	3		
Group I	898	829	87	17	42	33	650
Group II	459	422	28	15	23	24	332
Group III	721	608	74	16	30	14	474
Group IV	711	642	69	14	33	13	513

* 1 = dead germs the first 7 days of incubation
2 = dead germs between the 7th and 18th day of incubation
3 = dead germs between the 19th and 21st day of incubation

APPENDIX

TABLE VII

EFFECT OF DIET UPON FERTILITY AND HATCHABILITY OF NEW HAMPSHIRE PULLETS IN EXPERIMENT 2

DIET	NO. EGGS SET	NO. FERTILE EGGS	DEAD GERMS*			PIPPED EGGS	NO. CHICKS
			1	2	3		
Group V	459	407	59	18	22	25	283
Group VI	527	478	44	13	9	11	401

* Legend of dead germs, see Table III, page 78.

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ABSTRACT

In folic acid studies on egg production, fertility, and hatchability, 4 groups each of 15 New Hampshire pullets were maintained in batteries. Group I received a practical ration containing 33.8 micrograms of folic acid per 100 grams of ration (diet 1). Groups II and III were fed this diet supplemented with 200 and 400 micrograms, respectively, of synthetic folic acid per 100 grams of diet. Group IV received a typical all-purpose mash. No differences were noted in egg production, fertility, and hatchability of the 4 groups. Artificial insemination was employed. The progeny from the 4 groups appeared normal with respect to down color, weight, size, and viability.

In Group I diet when ground wheat was substituted for wheat middlings, the folic acid activity was lowered from 33.8 to 26.3 micrograms per 100 grams of diet. Consequently, additional folic acid was required to maintain normal egg production and hatchability. Fertility was not affected.

Mortality, growth, feather pigmentation, and feather development of progeny from the 4 groups were greatly influenced by maternal diet. In viability and growth studies, the mortality was the highest and the growth rate the slowest in chicks from Group I dams, irrespective of chick diet. Chick diets employed were: hen basal ration (diet 1) throughout 8 weeks; diet 1 during the first 4 weeks followed by a typical broiler mash (diet 4) the second 4 weeks; and diet 4 throughout 8 weeks.

Feather pigmentation and feather structure studies showed a high percentage of abnormal feather pigmentation in survivors from Group I dams, irrespective of the chick diet. All other progeny showed very little, if any, evidence of this abnormal condition on any chick diet. Males showed a greater percentage of abnormal feather pigmentation and poor feather development than females.

Addition of synthetic folic acid or Parvo, a crude folic acid supplement, was found to be the most effective supplement in reducing the degree of abnormal feather pigmentation. Fresh hen feces and dried cow manure showed little folic acid activity in this respect.

To observe the effect of the first 2-weeks' diet upon subsequent growth, with folic acid as the limiting factor, 3 series of experiments were conducted for a period of 6 weeks: series A, in which chicks were fed folic acid supplements beginning at hatching time; series B and C, in which chicks were fed diet 1 and diet 4, respectively, for the first 2 weeks, followed by folic acid supplements from the second to the sixth week. Chicks in series A produced a greater growth response than those in series B or C. The degree of abnormal feather pigmentation was greater in series B and C than in series A.

Incorporation of Parvo in a folic acid-low ration to supply at least 100 micrograms of folic acid per 100 grams of diet supported normal growth in New Hampshire chicks the first 4 weeks of life.