THE EFFECT OF CERTAIN NUTRIENTS AND OTHER MATERIALS UPON THE INCIDENCE OF BLOOD SPOTS IN CHICKEN EGGS

By

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AN ABSTRACT

Submitted to the School for Advanced Graduate Studies
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ABSTRACT

Eight experiments were conducted, using 456 pullets (strain-cross Single Comb White Leghorns or hybrids), to determine the effects of certain mineral deficiencies, excesses and imbalances; fat-soluble and water soluble vitamin levels; unknown growth factors; protein levels and nutrients that are concerned with blood and capillary nutrition on the incidence of blood spots in eggs. Certain materials related to atherosclerosis, such as cholesterol, were also fed to determine their effects. The incidence of blood spots of individual birds within a group, before and after a change in diet, was the criterion used to evaluate the dietary treatments employed.

The following dietary changes did not affect the incidence of blood spots: Higher than normal levels of vitamins A, D, E and K, cholesterol, i-inositol and betaine-HCl; lower than normal levels of sodium, chlorine, protein and vitamin D; abnormal balances of sodium and potassium or sodium and chlorine; and addition of arsanilic acid, corn fermentation solubles or Vigofac. The incidence of blood spots was significantly increased by the following: Diets with very high levels of vitamin B_{12}, zero or 2,156 USP units of vitamin A per pound. The effect of very high levels of vitamin B_{12} was counteracted with a very high level of vitamin A.

A consistent but non-significant reduction in the incidence of blood spots was obtained with diets containing very high levels of vitamin A.

A significant reduction in the incidence of blood spots was obtained on a diet containing five percent refined cottonseed oil.

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INTRODUCTION

The annual loss of market eggs due to blood spots ranges from two to four percent of the eggs produced. These eggs are rejected and do not get into market channels. Unfortunately, an equal or greater number of eggs containing small blood spots do reach the consumer. Since, from an esthetic viewpoint, consumers object to the presence of blood and meat spots in broken-out eggs, an even greater loss to the poultry industry probably results in terms of lowered consumption. Eggs with blood spots have equal nutritional value to those without blood spots.

The occurrence of blood spots has been studied by numerous investigators, but none, so far, have found a complete solution to the problem. Different individuals, species and strains of birds are known to vary somewhat in their dietary requirements for certain nutrients. It appeared feasible, therefore, to study the effect of excesses and deficiencies of certain important nutrients and other materials on the occurrence of blood spots.

The studies herein reported involved sodium and potassium imbalances, arsanilic acid, protein levels, vitamins A, B_{12}, E and K, inositol, betaine, unidentified growth factors, cholesterol, and fat. Of particular interest were those specific nutrients considered to be related to fragility of capillaries, blood clotting, and tissue integrity.

REVIEW OF LITERATURE

History and Importance

Aristotle (300 B.C., cited by Nalbandov and Card, 1944) believed that blood spots in eggs resulted from premature yolk expulsion. Fabricius, in 1600, used the occurrence of blood spots in eggs as proof of his theory that eggs were formed from blood. Not until the early 1930's did researchers show an interest in the problem of blood and meat spots in chicken eggs.

According to Quinn and Godfrey (1940), the incidence of blood spots in eggs varies significantly from one breed to another and within breeds. As evidence of this, ten Midwestern leghorn flocks were reported by Nalbandov and Card (1944) to have an incidence of 12.5 percent blood spots of which 7.7 percent were under one-eighth of an inch in diameter - too small to be detected by candling. In heavy breeds the incidence of blood and meat spots was found to be 41.7 percent. Merritt (1950) found that as high as 20 percent of the turkey eggs examined contained either blood or meat spots. White meat spots were found in 3.1 percent of the duck eggs broken out. Pheasant eggs contained 19 percent meat spots.

Dawson (1953, 1955) estimated that the loss to Michigan producers from blood spots exceeded $700,000 in 1953 and $1,000,000 in 1955.

Origin of blood spots

Follicular hemorrhages occuring at the time of ovulation have often been regarded as the cause of blood spots. However, Nalbandov and Card (1944) laparotomized hens and found that bleeding at the time of ovulation rarely occurs and is insignificant in amount. They found yolks with both small and large blood clots adhering to them even though no hemorrhaging had taken place at ovulation. After making numerous observations, these workers concluded that the bleeding was actually intrafollicular, and that it may have occurred several days before the release of the yolk. The hemorrhages may have occurred any place in the follicle. Large masses of blood diffusing between the yolk and follicle caused narrow, elongated masses of blood on the yolk; a wide sheet of blood was the result of a severe hemorrhage.

Origin of meat spots

Meat spots were described by Benjamin and Pierce (1937) as follows: "They may be grayish-white, in which case they are probably portions of loosened glands that have been torn from the wall of the oviduct as the egg passed through; or they may be red or flesh-color, in which case they are probably abnormal growths of oviduct and are later dislodged when an egg passes through."

Burmester and Card (1938) reported that 97 percent of the meat spots examined in their studies were of extravascular origin and that the degeneration of the red cells varied greatly among specimens of meat spots. In later work, Nalbandov and Card (1944) reported that blood spots degenerate into meat spots as a result of the alkaline pH of the egg white surrounding the clot. This degeneration of blood spots into meat spots was explained as follows: "Hemoglobin, when combined with oxygen, forms oxyhemoglobin, which has the bright red color characteristic of fresh blood. Under the influence of either an acid or a base, oxyhemoglobin breaks down into protein and either acid- or alkali-hematin, which has the characteristic yellowish or tan color of the typical meat spot. Further degeneration of the hematin will cause the nearly white meat spots often found in eggs."

In more recent work, Helbacka and Swanson (1958) reported that meat spots in white-shelled eggs were degenerated blood spots. Those in brown-shelled eggs were of two distinctly different types: (1) Those having their origin as blood spots and being non-fluorescent in ultra-violet light; or (2) those having their origin as bits of tissue of the oviduct. The latter contain considerable quantities of calcium and are fluorescent due to their porphyrin content.

Genetic relationship

Mention has already been made of the work of Quinn and Godfrey (1940) in which they reported significant breed and family differences in the incidence of blood spots. This was substantiated by Jeffrey (1945). In later work, Quinn *et al*. (1948) and Lerner *et al*. (1951) developed strains of Rhode Island Reds and Single Comb White Leghorns, respectively, which differed in their incidence of blood spot eggs. Positive selection appeared to be very effective but negative selection for a low incidence was not as effective.

Farnsworth and Nordskog (1955) estimated the heritability of blood spots to be 0.32 using a sample of 15 eggs per hen.

Physiological aspects

Many attempts have been made to correlate the incidence of blood spots with "physiological stress." The term "stress" or "stress factors" has come into use in the poultry language as if it were some new disease. It frequently is used as a nutritionist uses the term "unknown" factors to explain the occurrence of some undesirable condition that arises in a poultry flock. Since it has some unknown quality to its meaning, it would be well to establish what is meant by stress. The medical dictionary defines stress as "any stimulus or succession of stimuli of such magnitude as to tend to disrupt the homeostasis (stable normal condition) of the organism; when mechanisms of adjustment fail or become disproportionate or incoordinate, the stress may be considered an injury, resulting in disease, disability or death." In other words, stress is any condition which is adverse to the normal well-being of the individual or animal. Chickens, as well as other animals, are subject to stress. Many things can produce stress - season, physical disturbance, disease, etc.

Seasonal variation in the incidence of blood spots has been observed, but with little unanimity. Lerner and Smith (1942) found significant seasonal differences, with a definite increase after April 1st. According to Jeffrey (1945) the tendency is highest at the beginning of the laying year and decreases through the following August. Lerner and Taylor (1947) found an increasing incidence of blood spots until June, followed by a decrease through September. A similar, though not identical, trend was reported by Sharma (1949). Non-significant changes were reported by Denton (1947) and King and Hall (1955).

High intensity of production is sometimes considered a factor contributing to stress. However, Sauter _et al_. (1952) and Farnsworth and Nordskog (1955) stated that the intensity of production was not correlated with the incidence of blood spots. Likewise, Jeffrey (1945) and Sauter et al. (1952) noted that a pause in egg production had no effect on the incidence of blood spots. Farnsworth and Nordskog (1955) also concluded from their studies that the incidence of blood spots was not associated with sexual maturity or egg weight.

The extent to which physical disturbances affect the incidence of blood spots has been investigated by several groups. Jeffrey and Pino (1943) reported that frightening laying hens with dogs had no effect on blood spots. Stiles (1958) also found that physical disturbances (handling, sound and varying light intensities) did not affect the incidence.

Nutritional aspects

Deficiencies of certain essential nutrients have been reported to increase fragility of capillaries. Armentano et al. (1936) reported that "vitapric," an impure concentrate of ascorbic acid from Hungarian red pepper, gave favorable results in the treatment of cases of hemorrhagic purpura of the vascular type. Pure ascorbic acid failed to give this result. Upon subsequent fractionation of these peppers, these workers successfully isolated a highly active and almost pure flavone glucaside, called citrin or vitamin P. Nalbandov and Card (1944), however, reported that the addition of vitamin P to a practical laying ration did not affect the incidence of blood spots. These latter workers also failed to obtain any beneficial effect on blood spots from feeding high levels of vitamins A, C, D, E or K. They did, however, obtain a significant reduction in the incidence of blood spots when birds were given access to mixed grass range. Feeding of fresh grass, or access to dirt yards, failed to give a reduction in the incidence of blood spots. Denton (1947) reported that in his studies, the ingestion of green grass and range had no appreciable effect on the incidence of blood and meat spots - nor did vitamin K.

Carver and Henderson (1948) reported that the addition of rutin and vitamin C did not affect the incidence of blood spots, nor did high level (15 percent) feeding of dehydrated alfalfa leaf meal. Sauter et al. (1952), however, reported that hens receiving a diet containing 10 percent dehydrated alfalfa consistently produced a lower incidence of both large and small blood spots than did hens receiving a ration containing no alfalfa. In earlier unpublished work, Bearse (1940) also found an increased incidence of blood spots in eggs from hens fed a low level of alfalfa as compared with higher levels.

Bearse (1955) reported that hens receiving diets containing a suboptimal level of vitamin A produced eggs containing a higher incidence of blood spots than did those receiving 2000 USP units of vitamin A per pound of feed. He also stated that the results appeared to be linear - as the level of vitamin A in the diet was increased, the incidence of blood spots decreased. Birds on diets containing 553 USP units per pound produced at a rate of 43 percent of which 39.6 percent had blood spots; hens receiving diets containing 1,579 units produced at a rate of 63.3 percent of which only 16.1 percent contained blood spots. In subsequent studies, Bearse obtained further reduction in the incidence of blood spots by feeding very high levels of vitamin A (9,990 USP units per pound). In addition, favorable results were obtained on a diet to which had been added 3,000 USP units of vitamin A and 15 micrograms of vitamin B_{12} per pound. These results are not in agreement with those reported by Nalbandov and Card (1944) and Denton (1947). It is difficult to compare results further since the latter researchers did not furnish information on the diets used.

Inorganic arsenic (Frost, 1953) has a very favorable effect on growth and has had a long history in therapeutic use; therefore, related compounds may have a favorable effect on the occurrence of blood spots. For example, therapeutic use of potassium arsenite was first described in 1786 by Fowler (Frost, 1953). In work to establish the role of arsenic compounds in health, Gies (1877) reported that arsenic feeding stimulated bone marrow development and led to thickening of bones in rabbits, chickens and pigs. He also stated that a small level of arsenic led to improvement in the entire nutrition and appearance of the experimental animals. Arsanilic acid (p-amino-phenylarsonic acid) was reported to stimulate growth of poultry by Bird (1952) and to improve production of laying pullets without having any ill effects on hatchability or fertility in experiments reported by Libby et al. (1955). Carlson (1957), and Pope and Schaible (1958) reported that arsanilic acid was more effective in stimulating production in low-protein than in normal-protein diets.

Slovinsky (1948) examined 84 hypertensive patients and reported that 72 exhibited increased capillary fragility. Dividing these individuals into groups and orally administering either vitamin C, P or K did not appear to be superior to no treatment. Spontaneous recovery in the control group was as high as in any of the treated groups. This is not a suggestion that hypertension and capillary fragility are associated effects, but is mentioned since it is recorded that the two have occurred in the same patients.

Experimentally produced hypertension in hens has been reported by several workers. Krakower and Heino (1947), Lenel et al. (1948), and Weiss (1950) produced hypertension in birds by giving the birds solutions of saline in place of drinking water. Hypertension was produced in six-week-old chicks within two weeks by Lenel et al. (1948) and in laying hens by Weiss by supplying the birds with drinking water containing as little as 0.9 percent salt.

EXPERIMENTAL PROCEDURE

General

The 384 pullets used in the floor experiments were a strain-cross of Single Comb White Leghorns developed at Michigan State University. The pullets were hatched July 3, 1955, and reared on the starting and growing diets presented in Table 1. At housing time (December 15, 1955), the pullets were selected on the basis of body condition and freedom from obvious disease symptoms. They were then divided at random into twenty-four groups of sixteen birds each and placed in floor pens with wood shavings for litter.

The pens allowed 4.56 square feet of floor space per bird and were equipped with four trapnests, a dropping pit ($3\frac{1}{2}$' by 4') and one automatic drinking cup (Float type). The birds were given the basal diet A of Table 2 for fifteen days before being placed on their experimental regimes. During this pre-experimental period, as well as during all subsequent test periods, the pullets were trapnested, and the eggs were collected four times daily and placed in an egg cooler (60° F.) until they were broken out on the following day. Within 24 hours after an egg was laid, it was broken out on a glass plate with mirrors both underneath and behind to allow full observation of the eggs for blood spots.

After the completion of the pre-experimental period on December 30, 1955, one pen remained on basal diet A while all others were placed on the experimental diets presented in Tables 7 and 11.

Statistical Procedures

The results obtained on the incidence of blood spots during the experimental period were converted into percentage for each hen. Since

TABLE 1

DIETS USED FOR REARING PULLETS
(Amounts in percentages, except where indicated)

Ingredients	Diets Starter	Grower
Corn, grd. No. 2 yellow	45.00	45.00
Oats, grd. heavy	5.00	15.00
Wheat, grd.	--	10.00
Wheat bran	5.00	5.00
Wheat, std. middlings	5.00	5.00
Alfalfa leaf meal, dehyd. 17% prot.	5.00	5.00
Soybean oil meal, solv. 44% prot.	20.00	15.00
Meat and bone scraps, 50% prot.	5.00	2.50
Fish meal, Red	2.50	1.25
Whey, dried cheese	2.50	1.25
Yeast, dried brewer's	2.50	--
Limestone, grd.	1.50	1.25
Bone meal, steamed	1.00	1.50
Salt, iodized	.30	.50
Manganese sulfate, 70% feed. grade	.02	.013
Vitamin A and D feed. oil (2250A, 300D)	.25	.150
Choline chloride, 25% dry mix	.15	--
Pro-pen[1]	.005	.10
Pantothenate, calcium	--	200 mg

1 An antibiotic feeding supplement containing 2 grams of procaine penicillin and 3 mgs. of vitamin B_{12} per pound.

TABLE 2

COMPOSITION OF ALL BASAL DIETS
(Amounts in percentages, except where indicated)

Ingredients	Floor layers A	Floor layers B	Caged layers C
Barley, grd.	--	15.00	--
Corn, No. 2 yellow grd.	45.30	--	34.5
Wheat, grd.	10.00	10.00	--
Wheat bran	--	--	15.00
Wheat, flour middlings	5.00	25.00	10.00
Oats, heavy pulv.	15.00	20.00	20.00
Alfalfa meal, dehyd., 17% prot.	4.00	--	3.00
Soybean oil meal, solv., 44% prot.	10.00	10.00	2.50
Meat and bone scraps, 50% prot.	2.50	2.50	3.00
Fish meal, red	1.25	2.25	2.50
Whey, dried cheese	2.50	2.50	--
Milk, dried skimmed	--	--	2.00
Cottonseed oil	--	3.50	--
Sugar, Gran. cane	--	5.00	--
Limestone, grd. (98% $CaCO_3$)	1.25	1.25	5.00
Bone meal, steamed	2.50	2.50	1.50
Salt, iodized	0.50	0.35	0.60
Manganese sulfate, 70% feed grade	0.25	0.025	--
Vitamins per 100 lbs			
Choline chloride, 25% dry mix	--	0.15	--
Vitamin A & D feeding oil (2250A 300D)	0.35	--	0.53
Vitamin B_{12} suppl. (6 mg/lb)	10 gm	0.025	--
Vitamin suppl. 249C[1]	25 gm	--	--
Pantothenic acid	--	150 mg	--
Riboflavin	--	75 mg	--
Vitamin D_3 (3,000 ICU/gm)	--	25 gm	--
Menadione (8,000 mg/lb)	--	2 mg	--

1 Contains 2 grams of riboflavin, 4 grams of pantothenic acid, 9 grams of niacin and 10 grams of choline per pound in a fermentation residue carrier.

the distribution of blood spots was not definitely known, frequency distributions were made of the cumulative percentages of data from experiments conducted in floor pens during 1956, also the logarithms of these data were plotted on probability paper. The nature of this paper is such that normal distribution results in a straight line. The logarithms of the percentages of blood spots were analyzed using the least squares method of analysis (Snedecor, 1950).

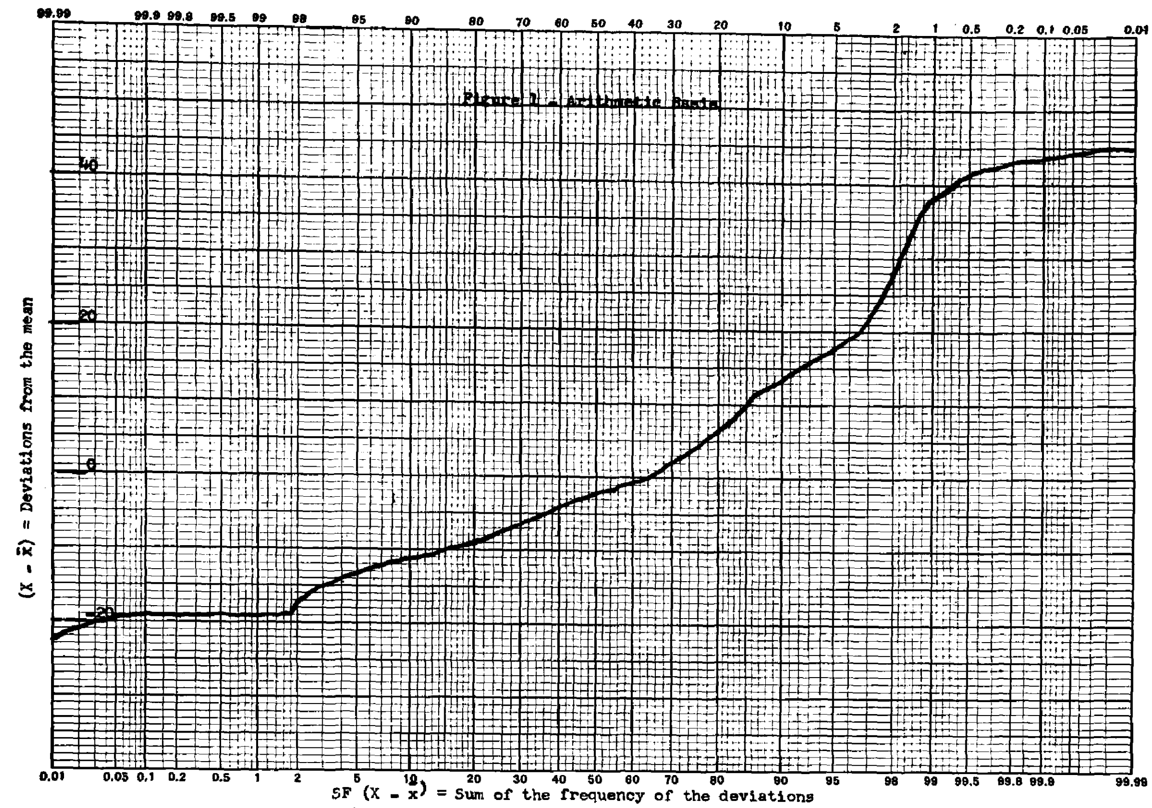
Handling the data in the above manner may be criticized from the standpoint that it does not take into consideration genetic variability. Consequently, the method of weighting data in statistical analysis reported by Baten and Henderson (1957) was used to compare the percentage of blood spots produced by a group of birds during a three-week period prior to receiving a particular experimental diet with that produced by the same birds between the sixth and ninth weeks on the experimental diet.

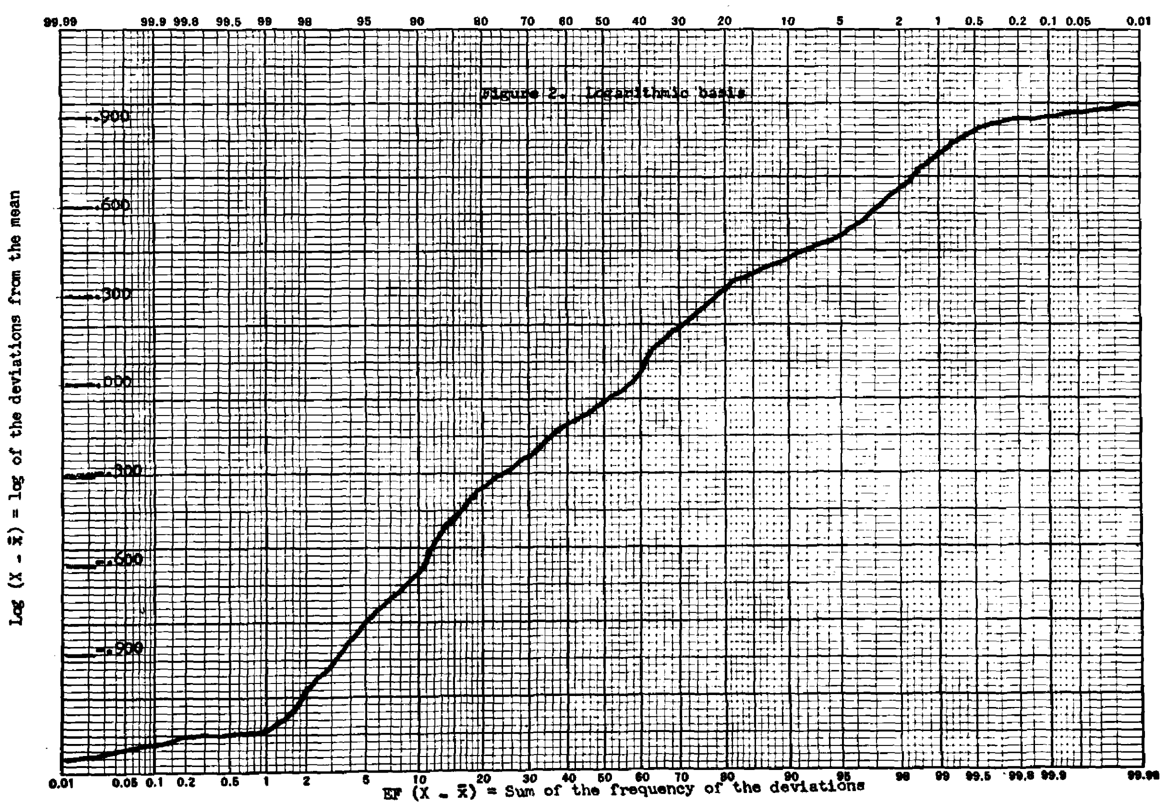
The hatchability data obtained in Experiment C were analyzed using the Chi-square method of analysis (Snedecor, 1950). Egg production data of Experiment C (Phase 2) were analyzed using the least squares method of analysis (Snedecor, 1950), and the means compared using Duncan's Multiple Range and Multiple F test (Duncan, 1955).

Statistical studies on the nature of blood spot data

The graphs obtained by plotting the percentages of blood spots and their logarithms (Experiments A through C) are presented in Figures I and II, respectively. The frequency distributions of the blood percentages are abnormal; whereas, that of the logarithms are more nearly normal.

DISTRIBUTIONS OF THE PERCENTAGE OF BLOOD SPOTS IN EXPERIMENTS A THROUGH C





Since the logarithms of the percentage of blood spots appeared to remove most of the skewness of distribution from these data, the cumulative results of Experiments A through C, as well as D and E were analyzed in this manner. These results are presented in Tables 3 and 4.

Analysis of these data (Tables 5 and 6) shows that the dietary treatments employed in these experiments had no significant effect on the incidence of blood spots. This was due, primarily, to the high degree of variability within treatments - the coefficient of variability was 87.11 percent for Experiments A through C and 39.16 percent for Experiments D and E.

If the feeding regime caused an increase or decrease in incidence, the change would be gradual in most cases. Thus, total incidence per bird per period would be a less sensitive indicator than that obtained toward the end of the regime. Consequently, incidences of blood spots were compared for a period three weeks before a change in diet and for a three week period starting at least six weeks after the birds had been on the new regime.

Due to these conditions, as well as genetic variability between hens and different rats of production, all succeeding statistical analyses were made by the method of Baten and Henderson (1957). This method takes into consideration individual variability, as well as discrepancies in rates of production.

TABLE 3

INCIDENCE OF BLOOD SPOTS PRODUCED BY PENS ON DIETS USED IN EXPERIMENTS A THROUGH C

Diet	Blood spots Log	Blood spots Antilog	Blood spots Percent of all eggs
A	0.640	4.4	6.0
1a	0.530	3.4	6.0
2a	0.292	2.0	3.6
3a	0.560	3.6	8.8
4a	0.728	5.4	9.0
5a	0.370	2.3	4.5
6a	0.806	6.3	9.6
7a	0.515	3.2	7.4
8a	0.447	2.8	5.6
9a	0.858	7.2	8.6
10a	0.523	3.3	3.5
11a	0.736	5.4	7.2
12a	0.523	3.3	2.3
13a	0.852	7.1	11.6
14a	0.584	3.8	8.0
15a	0.925	8.4	13.5
16a	0.843	7.0	11.5
17a	0.686	4.9	9.2
18a	0.534	3.8	12.8
19a	0.397	2.5	9.9
20a	0.580	3.8	13.4
21a	0.577	3.8	10.0
22a	0.600	4.0	9.7
23a	0.769	5.7	12.5
Mean	0.613		
S. E.	0.140		

TABLE 4

INCIDENCE OF BLOOD SPOTS PRODUCED BY PENS ON DIETS USED IN EXPERIMENT D AND E

Diet	Blood spots		
	Log	Antilog	Percent of all eggs
A	0.785	6.1	7.7
1b	0.659	4.6	6.4
2b	0.479	3.1	5.1
3b	0.785	6.1	13.2
4b	0.768	5.9	11.8
5b	0.351	3.2	5.1
6b	0.694	4.9	5.8
7b	0.420	2.6	4.0
8b	0.209	1.6	3.9
9b	0.879	7.6	11.3
11b	0.155	1.4	1.1
12b	0.252	1.7	1.7
Mean	0.588		
S. E.	0.079		

TABLE 5

ANALYSIS OF VARIATION OF BLOOD SPOT DATA OF EXPERIMENT A THROUGH C WITH REGARD TO CHANGES IN RATIONS*

Source of variation	Degrees of freedom	Mean square	F	F .05
Treatment	23	373,149	1.30	1.84
Error	320	284,985		

Mean of all treatments	0.613 ± 0.140**
Standard deviation	0.534
Coefficient of variability	87.110 percent

* Percentage data converted to logarithms
** Standard error of the mean

TABLE 6

ANALYSIS OF VARIATION OF BLOOD SPOT DATA OF EXPERIMENTS D AND E WITH REGARD TO CHANGES IN RATIONS*

Source of variation	Degrees of freedom	Mean square	F	F .05
Treatment	23	78,811	1.39	1.57
Error	233	52,998		

Mean of all treatments	0.588 ± 0.079**
Standard deviation	0.230
Coefficient of variability	39.16

* Percentage data converted to logarithms
** Standard error of the mean

EXPERIMENT A

The experimental diets (1a through 7a) presented in Table 7 were designed to study the effects of sodium and potassium "imbalances" in the diet on the incidence of blood spots.

These diets were modifications of basal diet A. As in all subsequent tests, ground yellow corn was used to balance out the variable amounts used.

Diets 1a and 2a replaced the iodized salt of the basal A with two levels of potassium chloride.

Diets 3a and 4a had salt and potassium chloride combinations at higher than usual levels. Diets 5a, 6a and 7a had increasing amounts of sodium carbonate added to the usual levels of NaCl.

Results

The incidence of blood spots produced by the different groups of birds in this experiment was relatively high, varying from 6.0 to 9.6 percent of the total eggs produced per pen (Table 8).

Three pens had an increase in incidence after a change in diet, three a decrease and one remained the same. Birds receiving the basal showed an increased incidence of blood spots. A significant increase, however, occurred only in the group (1a) where the NaCl was replaced by a low level of KCl. The degree of change in incidence on this particular diet was small compared to that for birds on diet 4a. This small change was found to be significant; whereas, the greater one was not. This is because the birds on diet 1a reacted more consistently than those on diet 4a during both periods. Furthermore, it is realized that relatively small numbers of eggs are involved in these comparisons. These two groups represent the extremes; therefore, detailed data for these are presented in Table 9.

It is seen that the use of more than the usual amount of total salt (NaCl, KCl or combinations of NaCl with KCl or $NaCO_3$), complete or partial removal of supplemental NaCl, increasing the amount of potassium alone or in relation to sodium or increasing the basicity of the ration with graded amounts of $NaCO_3$ did not significantly alter the blood spot incidence.

Egg production was satisfactory on the experimental diets; on the control diet, however, it was significantly lower than on experimental diets (Table 10). Examination of the data failed to reveal the reason for this. Actually, this control diet was used during 1957 (data not reported here) and supported satisfactory production.

Mortality was somewhat higher than normal for this short period but there was no apparent association with respect to changes made in the rations.

TABLE 7

MINERAL CHANGES MADE IN BASAL DIET A IN EXPERIMENT A
(Amounts in percentages)

Ingredients	Diets							
	Basal A	1a	2a	3a	4a	5a	6a	7a
NaCl, iodized	0.5	--	--	0.5	0.25	0.5	0.5	0.5
KCl[1]	--	0.1	0.5	0.5	0.75	--	--	--
$NaCO_3$[1]	--	--	--	--	--	0.1	0.3	0.5

1 USP grade chemicals

TABLE 8

EFFECT OF CERTAIN MINERALS ON PERFORMANCE DURING PERIODS BEFORE AND AFTER CHANGES IN RATIONS
(16 birds started per treatment)

Diets	Blood spots: 3 weeks before change (%)	Blood spots: Last 3 weeks after change (%)	$\bar{d}$	t	Degrees of freedom	After ration changes: Blood spots (%)	After ration changes: Mortality (%)	After ration changes: Av. egg production[1]
Basal A	1.4	3.1	.045	2.00	6	6.0	25.0	45.9
1a	4.7	6.4	.046	3.58**	9	6.0	31.2	76.0
2a	5.9	2.4	.081	1.88	8	3.6	25.0	77.5
3a	11.3	12.3	.034	.74	11	8.8	12.5	76.3
4a	12.3	3.4	.027	1.04	6	9.0	31.2	68.2
5a	4.9	7.9	.016	.55	6	4.5	25.0	71.2
6a	4.5	4.5	.000	.00	6	9.6	18.7	80.8
7a	6.0	1.5	.080	1.51	8	7.4	25.0	79.3

1 Production during the 112-day experimental period.
** Significant $P < .01$

TABLE 9

INCIDENCE OF BLOOD SPOT EGGS AND EGG PRODUCTION ON AN INDIVIDUAL BASIS FOR CERTAIN GROUPS IN EXPERIMENT A FOR A 3-WEEK PERIOD BEFORE AND AFTER DIETARY CHANGE

Hen	Diet 1a Before eggs	Diet 1a Before spots	Diet 1a After eggs	Diet 1a After spots	Hen	Diet 4a Before eggs	Diet 4a Before spots	Diet 4a After eggs	Diet 4a After spots
1	7	0	18	1	1	8	3	14	1
2	10	1	18	1	2	12	2	17	0
3	4	1	22	1	3	8	0	17	0
4	12	0	20	1	4	2	0	18	0
5	2	0	19	1	5	10	2	16	1
6	12	0	19	1	6	10	0	20	2
7	13	0	15	0	7	7	0	17	0
8	14	0	19	1					
9	11	2	19	4					
10	1	0	19	1					
Total	86	4	188	12		57	7	119	4
Percent		4.7		6.4			12.3		3.4
Change				+1.7					-8.9
$\bar{d}$				.046					.027
t				3.58					1.04
t P = 0.05				2.26					2.45
t P = 0.01				3.25					3.71

TABLE 10

ANALYSIS OF VARIANCE OF EGG PRODUCTION DATA AS AFFECTED BY MINERAL CHANGES IN THE DIET

(A) Analysis of variance

Source of variation	Degrees of freedom	Mean square	F	F .05
Treatments	7	1467	3.17	2.69
Error	89	462		

(B) Least significant ranges (P = 0.01)

Diet	A	4a	5a	1a	3a	2a	7a	6a
Mean	45.9	68.2	71.2	76.0	76.3	77.5	79.3	80.8

EXPERIMENT B

The experimental diets presented in Table 11 were employed to study the effects of vitamins and unidentified factors on the incidence of blood spots. The latter were included since Donovan et al. (1958) and Camp et al. (1957) have shown that chicks require a factor of factors contained in certain fermentation residues.

Diets 8a through 13a had increasing levels of vitamin B_{12}, A and D_3. In diets 8a through 10a, vitamin B_{12} was at a level of thirty micrograms per pound of feed; in diets 11a through 13a, it was at a level of sixty micrograms. At both levels of vitamin B_{12}, vitamin A was added at levels of 3,575 and 10,215 USP units per pound. Vitamin D was added at levels of 477 and 1,362 ICU per pound in diets 8a and 9a, as well as 11a and 12a. None was added to diets 10a and 13a.

In the remaining diets in Table 4, part of the NaCl was replaced with KCl (14a through 17a). All four of these diets contained high levels of vitamin B_{12} and vitamin A. Two of them (14a and 15a) contained different levels of corn fermentation solubles; whereas the other two (16a and 17a) contained different levels of Vigofac. Diet 18a contained a high level of vitamin E. Diet 19a contained high levels of vitamins A, D, E, K and B_{12} and unidentified "growth" factor sources, with the iodized salt being replaced by a low level of potassium chloride. Diets 10a and 13a through 17a did not contain vitamin D_3 and were, therefore, vitamin D deficient until March 14, 1956, when 120 International Chick Units of vitamin D_3 was added per pound in the form of an irradiated animal sterol feeding supplement containing 3,000 ICU units per gram.

TABLE 11

DIETARY TREATMENTS USED IN EXPERIMENT B
(Amounts in percentages, except in parenthesis where it is in grams)

Ingredients	Diets: Basal A	8a	9a	10a	11a	12a	13a	14a	15a	16a	17a	18a	19a
Salt	.5	.5	.5	.5	.5	.5	.5	.25	.25	.25	.25	.5	--
KCl (USP)	--	--	--	--	--	--	--	.25	.25	.25	.25	--	.1
Vitamins:													
B_{12}[1]	(10)	.25	.25	.25	.5	.5	.5	.25	.25	.25	.25	(10)	.25
A & D oil[2]	.35	.35	1.00	*	.35	1.0	*	*	*	*	*	.35	.35
A[3]	--	--	--	(10)	--	--	(10)	(10)	(10)	(10)	(10)	--	(10)
Myvamix[4]	--	--	--	--	--	--	--	--	--	--	--	2.0	2.0
K[5]	--	--	--	--	--	--	--	--	--	--	--	--	1.2
CFS[6]	--	--	--	--	--	--	--	1.0	5.0	--	--	--	1.0
Vigofac[7]	--	--	--	--	--	--	--	--	--	1.0	5.0	--	1.0

(1) 6 mg per pound; (2) 2250 USP units of A and 300 IC units of D_3 per gram; (3) gelatin stabilized palmitate form, with a potency of 250,000 USP units per gram; (4) 20,000 IU of vitamin E per pound; (5) 8 grams of menadione bisulfite per pound; (6) Corn fermentation solubles (Clinton Corn Proc. Co.; (7) Antibiotic fermentation residue (Pfizer).

* No vitamin D until March 14, 1956, after which it was added at a level of 120 ICU per pound.

Results

The percentage of blood spots produced during the period the birds were on experimental diets varied from about two to 13 percent for the different lots (Table 12).

Lots receiving high levels of vitamins B_{12} and A (12A), as well as a combination of low salt with high levels of a number of vitamins, showed an insignificant reduction in numbers of blood spots.

The incidence of blood spots was increased significantly in lots receiving diets 8a and 11a. Both of these diets contained ten or twenty times the normal amount of added vitamin B_{12}, respectively. They had normal levels of NaCl and vitamins A and D. High levels of vitamin A or vitamins A and D in diets containing high levels of vitamin B_{12} (9a, 10a, 12a) appeared to mitigate the deleterious effects of the high levels of vitamin B_{12}. Blood spot production was increased on diets containing corn fermentation solubles and Vigofac (14a to 17A). These differences were not significant, primarily due to the degree of variability between individual hens and the lowered rates of production obtained on these diets.

Vitamin D depletion did not significantly affect the incidence of blood spots produced, although the diets contained high levels of vitamin B_{12} and A, or A and unidentified "growth" factors.

Analysis of the egg production data (Table 13) indicates that birds on vitamin D-deficient diets produced significantly fewer eggs than the other groups. A high level of Vigofac helped to restore normal egg production - probably because of its high ash content which should reduce vitamin D requirements. Lack of vitamin D caused an obvious reduction in production by the seventh week of the experiment. After vitamin D_3 was added to the respective diets (March 14, 1956), production increased gradually over a five-week period to equal that obtained on other diets at that time. High levels of vitamin A, B_{12} or unidentified "growth" factors did not facilitate nor retard development of deficiency symptoms when added to rations deficient in vitamin D. This observation is based on day-by-day comparisons of production records of the individual pens concerned. Since production was highest in the groups receiving high levels of vitamin E, it would appear that the diets containing no added vitamin E may be bordering on a deficiency.

TABLE 12

EFFECT OF VITAMINS A, D, E, K, AND B_{12} AND UNIDENTIFIED "GROWTH" FACTORS ON BLOOD SPOT INCIDENCE, MORTALITY AND EGG PRODUCTION (16 birds started per group)

Diets	Blood spots: 3 wks. before change %	Blood spots: Last 3 wks. after change %	$\bar{d}$	t	Degrees of freedom	After ration changes: Blood spots %	After ration changes: Mort-ality %	After ration changes: Av. egg production[1]
Basal A	1.4	3.1	.045	2.00	6	6.0	25.0	45.9**
8a	4.5	10.2	.083	4.15**	7	5.6	0.0	55.3
9a	7.8	12.9	.061	2.35	5	8.6	37.5	55.0
10a	1.4	2.6	.034	.12	8	3.5	12.5	33.7**
11a	4.7	12.7	.098	4.26*	4	7.2	6.7	53.7**
12a	6.3	2.8	.001	.06	10	2.3	12.5	59.1
13a	5.3	10.7	.002	.00	7	11.6	31.3	25.5**
14a	2.4	5.3	.027	.22	8	8.0	25.0	32.3**
15a	6.4	16.3	.015	.29	11	13.5	6.7	44.9**
16a	18.6	32.4	.110	.58	9	11.5	12.5	37.2**
17a	4.0	13.5	.111	2.05	7	9.2	18.8	51.5
18a	8.5	2.2	.096	.20	9	12.8	18.8	59.6
19a	6.2	5.2	.009	.13	4	9.9	25.0	62.2

1 Production during the 112-day experimental period - all groups compared to 19a
* Significant $P<0.05$
** Significant $P<0.01$

TABLE 13

ANALYSIS OF VARIANCE OF EGG PRODUCTION AS AFFECTED BY VITAMINS A, D, E, K AND B_{12} AND UNIDENTIFIED "GROWTH FACTORS

(A) Analysis of variance

Source of variation	Degrees of freedom	Mean square	F	F .05
Treatment	11	2179	5.1	2.37
Error	154	427		

(B) Least significant ranges P = 0.05 and 0.01

Diet	13a	14a	10a	16a	15a	17a	11a	9a	8a	12a	18a	19a
Mean	25.5	32.3	33.7	37.2	44.9	51.5	53.7	55.0	55.3	59.1	59.6	62.2

P = 0.05

P - 0.01

EXPERIMENT C

The goal of this experiment was to determine the effect of arsanilic acid on the incidence of blood spots with pullets on high- and low-protein breeder diets. Each diet in Table 14 was fed to single groups of 16 pullets from January 1 to June 30, 1956.

Results

The results obtained on the incidence of blood spots are presented in Table 15. A statistical analysis of these data indicates that lowering the crude protein content of the diet from 16.8 to 13.1 percent had no effect on the incidence of blood spots produced. Further, arsanilic acid did not significantly affect the incidence of blood spots by the hens on either diet (Table 15).

TABLE 14

COMPOSITION OF BREEDER DIETS USED IN EXPERIMENT C
(Amounts in percentage except where indicated)

Ingredients	Low protein		High protein	
	20a	21a	22a	23a
Corn, grd. yellow	68.15	68.15	58.15	58.15
Oats, pulv. heavy	10.00	10.00	10.00	10.00
Alfalfa meal, dehyd., 17% prot.	5.00	5.00	5.00	5.00
Soybean oil meal, solv., 44% prot.	5.00	5.00	15.00	15.00
Fish meal, red	5.00	5.00	5.00	5.00
Bone meal, steamed	4.00	4.00	4.00	4.00
Limestone, grd.	2.00	2.00	2.00	2.00
Feeding oil (2250A 300D)	0.25	0.25	0.25	0.25
Salt, iodized	0.50	0.50	0.50	0.50
Vitamin Suppl. 249C	0.10	0.10	0.10	0.10
Vitamin B_{12} Suppl. (6 mg/lb)	12 gm	12 gm	12 gm	12 gm
Manganese sulfate, 70% feed grade	12 gm	12 gm	12 gm	12 gm
Pro-Gen[1]	--	22.7 gm	--	22.7 gm

1 Contains 20 percent arsanilic acid, Abbott Laboratories, North Chicago, Ill.

TABLE 15

EFFECT OF ARSANILIC ACID IN NORMAL- AND LOW-PROTEIN DIETS ON THE INCIDENCE OF BLOOD SPOTS PRODUCED (16 birds started per group)

Diet	3 wks. before change %	Last 3 wks. after change %	$\bar{d}$	t	Degrees of freedom	Blood spots after change of diet
20a	11.0	15.3	.0004	.25	7	13.4
21a	11.6	9.7	.0060	.26	7	10.0
22a	2.9	2.3	.0280	.22	5	9.7
23a	6.6	9.6	.0030	.43	9	12.5

EXPERIMENT D

After April 22, 1956, the pens which had previously received diets 1a through 7a were placed on a 3^2 factorial experiment to determine the effects of corn fermentation solubles, Vigofac and vitamin E on the incidence of blood spots. The design is shown in Table 16.

Results

Birds receiving basal diet A or the basal plus vitamin E in combination with corn fermentation solubles (5b) or Vigofac (6b) exhibited slight reductions in blood spots, while the remaining groups had increased incidence of blood spots (Table 17). The changes, however, were significant only for the groups receiving high levels of vitamin E (3b), Vigofac and corn fermentation solubles (4b) and combinations of the three (7b).

The increased incidence of blood spots on diets containing the high levels of vitamin E, corn fermentation solubles and Vigofac were not consistent because other diets which contained these ingredients did not show this trend. This finding, together with the relatively small numbers involved, makes it appear that chance or some uncontrolled factor was responsible for the differences.

Although the variations in the average rates of production were found to be significant at the five percent level of probability (Table 18), this is not considered adequate deviation from the control with respect to egg production to attribute special merits to any of the additives employed in this experiment.

TABLE 16

DIETARY TREATMENTS USED IN EXPERIMENT D
(amounts in percentages)

Ingredients	Diets							
	Basal A	1b	2b	3b	4b	5b	6b	7b
CFS	--	--	--	--	1.0	1.0	--	1.0
Vigofac	--	--	1.0	--	1.0	--	1.0	1.0
Myvamix	--	--	--	2.0	--	2.0	2.0	2.0

TABLE 17

EFFECT OF VITAMIN E, CORN FERMENTATION SOLUBLES, AND VIGOFAC AND COMBINATIONS ON THE INCIDENCE OF BLOOD SPOTS
(14 birds started per group)

Diets	Blood spots: 3 wks. before change %	Blood spots: Last 3 wks. after change %	$\bar{d}$	t	Degrees of freedom	After ration changes: Blood spots %	After ration changes: Mortality %	After ration changes: Av. egg production[1]
A	5.7	4.1	.030	.40	7	7.7	9.1	30.6
1b	4.3	6.7	.007	.96	9	6.4	0.0	49.4
2b	2.5	2.5	.046	.81	8	5.1	0.0	37.7
3b	10.9	13.6	.040	5.00**	13	13.2	0.0	47.7
4b	5.9	11.2	.056	4.31**	7	11.8	9.1	42.2
5b	9.0	7.8	.015	.24	5	6.1	25.0	42.1
6b	8.0	7.3	.001	.02	8	5.8	7.7	29.9
7b	1.3	5.2	.065	3.82**	9	4.0	0.0	43.2

1 Production during the 63-day experimental period

** Significant change $P < .01$

TABLE 18

ANALYSIS OF VARIANCE FOR THE EFFECTS OF VITAMIN E, VIGOFAC AND CORN FERMENTATION SOLUBLES ON EGG PRODUCTION

Source of variation	Degrees of freedom	Mean square	F	F .05
Treatment	7	606	2.56	2.12
Error	83	236		

EXPERIMENT E

Since preliminary data were not in agreement with published data, it was decided to use a basal diet composed primarily of "common" feed ingredients which would be very low in vitamin A. This basal diet (B) was fed to the pen which had previously received a diet (8a) with a very high level of vitamin B_{12}. Vitamin A in the palmitate form was then added to basal diet B at graded levels as shown in Table 19.

These diets were designated 9b, 11b, and 12b, respectively to facilitate reference to previous dietary treatments.

Results

The results of this study are presented in Table 20. Significant reductions in blood spots were obtained with the basal diet (B) containing no added vitamin A, as well as with those having high levels of vitamin A (11b and 12b). The birds on diet B and those on diet 11b had previously been on diets containing very high levels of vitamin B_{12}, which increased the incidence of blood spots.

A comparison of the incidence of blood spots in eggs laid by birds on these diets with incidence of spots in eggs laid by the same birds prior to the beginning of this experiment showed a significant change in the incidence of blood spots only for those birds receiving the highest level (9,000 USP units) of vitamin A per pound of feed (12b). This is 4.5 times the requirement (Bird et al., 1954).

The vitamin A-deficient diet (B) did not cause an increase in the incidence of blood spots during the relatively short duration of the experiment.

Variations in average rate of egg production were found not to be significant (Table 21).

TABLE 19

DIETARY TREATMENTS USED IN EXPERIMENT E. (Addition in pounds to 100 pounds of basal diet B)

Ingredients	Diets			
	Basal B	9b	11b	12b
Vitamin A palmitate*	--	0.05	0.10	0.20

* Gelatin-stabilized product in solvent-extracted, soybean oil meal. (10,000 USP units per gram)

TABLE 20

EFFECT OF VITAMIN A ON THE INCIDENCE OF BLOOD SPOTS

		Blood spots					After ration changes	
Diets	No. of birds	3 wks. before change %	Last 3 wks. after change %	$\bar{d}$	t	Degrees of freedom	Blood spots %	Av. egg production[1] %
Basal B	16	9.8	3.1	.145	3.54*	6	3.9	28.2
9b	15	9.9	10.0	.063	1.37	5	11.3	34.3
11b	12	14.5	3.4	.094	2.77*	4	1.1	26.8
12b	14	2.2	0.8	.053	8.83**	7	1.7	34.2

1 Production during the 63-day experimental period

* Significant change $P<0.05$

** Significant change $P<0.01$

TABLE 21

ANALYSIS OF VARIANCE OF EGG PRODUCTION ON DIETS DEFICIENT AND ADEQUATE IN VITAMIN A

Source of variation	Degrees of freedom	Mean square	F	F .05
Treatment	3	131	0.42	2.90
Error	32	315		

EXPERIMENT F

The purpose of this experiment was to study the effect of iodized salt, fat, cholesterol, inositol and betaine HCl on the incidence of blood spots. These rations were fed to pullets in a multiple-deck laying battery. The pullets were selected from the same group as those used in the floor experiments A through E. The birds were individually typed for incidence of blood spots for a five-month period. They were then placed on the dietary treatments presented in Table 8 using basal diet C - Table 22. Eight pullets were placed on each diet, except for diets 4c and 5c, where two pullets were used, and 6c where four pullets were used.

This experiment was started October 4, 1955,and birds were placed on experimental diets from February 20, 1956,until June 30, 1956.

Results

The incidence of blood spots produced by the birds in this experiment was quite low (Table 23). The analyses of the egg production are presented in Table 24.

The birds receiving a diet containing six percent cottonseed oil (3c) exhibited the only significant reductions in blood spots and egg production.

Lack of salt, or three times as much as needed, failed to modify the incidence of blood spots or influence the rate of production.

High level feeding of cholesterol, i-inositol or betaine HCl also did not effect the incidence of blood spots.

TABLE 22

DIETARY TREATMENTS USED IN EXPERIMENT F. (Amounts in percentages except where indicated)

Ingredients	Diets						
	Basal C	1c	2c	3c	4c	5c	6c
Salt, iodized	0.6	--	1.5	0.6	0.6	0.6	0.6
Oil, cottonseed	--	--	--	6.0	--	--	--
Cholesterol	--	--	--	--	400 gm	--	--
i-Inositol	--	--	--	--	--	200 gm	--
Betaine HCl	--	--	--	--	--	--	450 gm

TABLE 23

EFFECT OF SALT, COTTONSEED OIL, CHOLESTEROL, I-INOSITOL, AND BETAINE HCl ON THE INCIDENCE OF BLOOD SPOTS AND EGG PRODUCTION[1]

Diets	No. of birds	Blood spots: 3 wks. before change %	Blood spots: Last 3 wks. after change %	$\bar{d}$	t	Degrees of freedom	After ration changes: Blood spots %	After ration changes: Av. egg production[2]
C	8	5.2	7.7	.033	2.20	6	7.9	73.0
1c	8	5.5	5.7	.005	.24	7	5.2	78.9
2c	8	4.9	3.2	.024	.34	6	3.4	61.1
3c	8	4.8	2.4	.124	3.54	6	2.7	30.7*
4c	2	2.0	2.9	.009	.13	1	2.9	69.5
5c	2	5.7	5.0	.007	.38	1	5.0	89.5
6c	4	4.1	2.4	.006	.75	4	2.5	51.6

1 No mortality was encountered in this study

2 Average number of eggs laid during the 123-day experimental period

* Significantly low ($P < .05$) when compared to diets C, 1c and 2c

TABLE 24

ANALYSIS OF VARIANCE OF EGG PRODUCTION FOR EXPERIMENT F[1]

(A) Analysis of variance

Source of variation	Degrees of freedom	Mean square	F	F .05
Treatment	3	3,407	5.15	4.60
Error	27	662		

(B) Least significant ranges (P = 0.05 and 0.01)

Diet	3c	2c	C	1c
Mean	30.7	61.1	73.0	78.9

P = 0.05

P = 0.01

1 The production data for the pens receiving diets 4c, 5c and 6c were not used in this analysis because of small numbers involved.

EXPERIMENT G

Since preliminary observations of the data revealed that vitamin A did not appear to be as effective in lowering the incidence of blood spots as had been reported, the following experiments were initiated. A high level of vitamin A was added to a caged layer diet and fed to hens having a known tendency to produce large numbers of blood spots. The calculated vitamin A content of the basal diet, on the basis of corn, alfalfa, and A and D oil, was 8,067 USP units per pound.

A group of 28 birds was selected from 2,000 pullets (a commercial hybrid hatched April 4, 1957). These pullets were reared in the same manner as previously described. The pullets were selected on the basis of incidence of blood spots produced from September 18 until December 5, 1957. Only birds maintaining a rate of production greater than 60 percent and having an incidence of blood spots no less than 15 percent were used in the study. The selected pullets were moved from floor pens (previously described) and placed in multiple-deck laying batteries and given the dietary treatments starting December 5, 1957. The pullets were placed in individual cages. Half of them were fed Basal diet C. The other half were fed this ration plus 4,000 USP units of vitamin A palmitate per pound of feed. The source of the added vitamin A was a gelatin-stabilized product having a potency of 10,000 USP units of vitamin A per gram. The carrier was solvent extracted soybean oil meal containing 44 percent protein. This experiment was concluded February 28, 1958.

Results

The incidence of blood spots in both groups was lowered during the experimental period but the changes were not significant (Table 25). The vitamin A content of Basal diet C was calculated to be 8,067 USP units per pound which was well over the National Research Council requirement of 2,000. The additional vitamin A added brings the total to 12,067 units or several times the estimated requirement. It was apparent, therefore, that vitamin A, by itself, did not correct the defect.

The rates of production were extremely uniform within each group and there were no differences due to dietary treatment (Table 26).

TABLE 25

EFFECT OF HIGH AND NORMAL DIETARY LEVELS OF VITAMIN A ON THE INCIDENCE OF BLOOD SPOTS AND EGG PRODUCTION IN CAGED LAYERS
(14 birds started per group)

Diet	Blood spots: 3 wks. before change %	Blood spots: Last 3 wks. after change %	$\bar{d}$	t	Degrees of freedom	After ration changes: Blood spots %	After ration changes: Av. egg prod.[1]
C	15.19	5.71	.094	.87	12	10.85	49.4
C + vit. A[2]	11.28	7.53	.024	.45	8	9.11	50.7

1 Production during the 63-day experimental period
2 One bird in this group died twenty days after the experiment started.

TABLE 26

ANALYSIS OF VARIANCE OF EGG PRODUCTION DATA FOR EXPERIMENT G

Source of variation	Degrees of freedom	Mean square	F	F .05
Treatment	1	12	.09	4.17
Error	25	133		

EXPERIMENT H

This study was undertaken to find out the effects of high, practical and low levels of vitamin A on the incidence of blood spots produced by birds having a history of laying eggs with a high or low incidence of blood spots. The calculated vitamin A content, on basis of corn, alfalfa and A and D oil, was 8,067, 2,150 and 0 USP units per pound for diets C, D and E, respectively.

The 27 surviving birds from the previous experiment, and 9 other pullets having a blood spot incidence between 10 and 14 percent, were placed on basal diet C until April 16, 1958. In addition, 36 birds of the same age, breeding and rearing but with an incidence of only 10 percent or less of blood spots during the pre-experimental period were used.

On April 16, 1958, the two groups were subdivided into triplicate lots of 12 birds each, and placed on each of the diets, C, D and E (Table 27), respectively.

Diet C was fed to birds on the lower deck of the batteries, diet D to birds in the middle deck and diet E to those on the top level. This procedure was followed to eliminate the possibility of birds on the low-vitamin A diet from receiving vitamin A by accident. This experiment was conducted for nine weeks.

At the end of this experiment, all birds were sacrificed for liver and blood samples. The blood samples were taken by heart puncture, prior to killing the birds. The procedure was as follows: 0.5 ml of a heparin solution containing 300 milligrams percent of heparin

TABLE 27

DIETS USED IN EXPERIMENT H
(Amounts in percentages except where indicated)

Ingredients	Diets C	D	E
Corn, white, grd.	--	34.5	34.5
Corn, yellow, grd.	34.5	--	--
Oats, heavy pulv.	20.0	20.0	20.0
Wheat bran	15.0	15.0	17.5
Wheat, flour middlings	10.0	10.0	10.0
Alfalfa meal, dehyd., 17% prot.	3.0	3.0	--
Meat and bone scraps, 50% prot.	3.0	3.0	3.0
Milk, dried skim	2.0	2.5	2.5
Soybean oil meal, solv., 44% prot.	2.5	2.5	2.5
Fish meal, red	2.5	2.5	2.5
Limestone, grd. (98% $CaCO_3$)	5.0	5.0	5.0
Bone meal, steamed	1.5	1.5	1.5
Salt, iodized	0.6	0.6	0.6
Vitamin A & D feeding oil (2250A, 300D)	0.53	--	--
Stabilized choice yellow grease	--	--	0.53
Solka Floc (cellulose)	--	--	0.5
Menadione (8000 mg/lb)	--	--	1.2 gm
Myvamix (20,000 IU/lb)	--	--	6.0 gm

was placed into each centrifuge tube. The tubes were then dried in a forced draft oven at 60° C. for an hour. Thus, each 5 ml blood sample contained 1.5 milligrams of heparin. The blood samples were kept on ice and centrifuged within the hour at 1800 r.p.m. for 15 minutes. Plasma samples were removed and quick-frozen in a freezer chest (10° F) until the sample could be assayed for vitamin A content. The liver samples were taken immediately after bleeding, placed on ice and taken to a quick-freeze room (-40° F) within 30 minutes after the bird was killed.

The vitamin A content of the plasma was determined colorimetrically, using the method developed by Sobel and Snow (1947). In their procedure, one ml of plasma was saponified with alcoholic KOH at 60° C for 20 minutes. The non-saponifiable fraction was then taken up in petroleum ether. The petroleum ether was then evaporated off in a nitrogen atmosphere to prevent destruction of vitamin A. The small droplets of vitamin oil thus obtained were taken up in chloroform and diluted with activated glycerol dichlorohydrin (1-3, dichloro-2-propanol) to develop a pink color. A special horizontal absorption cell providing a 50 mm light path was used. To eliminate possible interference, a special light filter (555 millimicrons) was used in place of the standard wide-range filter supplied with the instruments. Readings were taken using a Coleman Model 14 spectrophotometer at a wave length of 550 millimicrons. Vitamin A was calculated in micrograms percent using a standard curve which had been derived under the above conditions with a Bureau of Standards vitamin A standard.

The vitamin A of liver samples was isolated using the method of Gallup and Hoefer (1946). The procedure was basically the same as that previously described, except for the amounts of reagents involved. Once the vitamin A was extracted and the ether evaporated in the presence of a nitrogen atmosphere, quantitative determinations were made using the glycerol dichlorohydrin reagent rather than Carr-Price reagent employed by Gallup and Hoefer. The latter procedure was used since Sobel and Snow (1947) showed that the activated glycerol dichlorohydrin procedure was more quantitative and less variable.

Results

The incidence of blood spots varied from 7 to 14 percent in the different lots. Diets D and E, which contained low levels of vitamin A, significantly increased the incidence for the "low-blood-spot" hens (Table 28) with only a slight increase for "high-blood-spot" hens.

On the basis of previous dietary history, the data for the "high-blood-spot" hens were then regrouped (Table 29). When considered in this manner, birds which had previously received 12,067 USP units of vitamin A per pound showed a decreased incidence on diets C and D; whereas, those which had previously received 8,067 units showed an increase. This increase in the group receiving 8,067 units was similar to the results obtained with the "low-blood-spot" hens which had previously been fed in a similar manner. All groups showed an increase on diet E regardless of previous dietary level of vitamin A.

Vitamin A determinations in duplicate were made by the method of Sobel and Snow (1947) on liver samples. Duplicate samples agreed; the

TABLE 28

EFFECT OF HIGH, NORMAL AND LOW VITAMIN A DIETS ON THE INCIDENCE OF BLOOD SPOTS[1]
(12 birds started per treatment)

Diet	Vitamin A level in diet	Previous blood spot incidence of hens	Blood spots: 3 wks. before treatment %	Blood spots: Last 3 weeks on treatment %	$\bar{d}$	t	After ration changes: Blood spots	After ration changes: Av. egg prod.[2]
C	High	High	15.4	15.1	.040	.53	14.7	37.4
C	High	Low	8.9	10.1	.022	.49	9.2	36.7
D	NRC[3]	High	12.1	7.6	.042	2.47	12.7	42.0
D	NRC	Low	7.3	16.5	.163	6.03**	7.8	28.9
E	Low	High	11.3	16.3	.009	.00	7.1	35.2
E	Low	Low	4.3	15.8	.152	4.61**	10.6	38.0

1 No mortality was encountered in this experiment
2 For the 63-day experimental period
3 National Research Council required level
** Significant change, $P < 0.01$, with 11 degrees of freedom.

TABLE 29

DIFFERENCES IN BLOOD SPOT INCIDENCE OF "HIGH BLOOD SPOT" HENS WITH RESPECT TO PREVIOUS AND LATER VITAMIN A DIETARY LEVELS

Experimental diets		Pre-experimental vitamin A units per pound	
No.	Vitamin A units per pound	8067 %	12,067 %
C	8067	+ 5.2	- 4.5
D	2156	+ 3.5	- 9.6
E	0	+ 7.2	+15.3**

1 Calculated on basis of corn, alfalfa and A and D feeding oil

** Significant $P < .01$

maximum difference between two samples from the same bird being five percent. It was possible to obtain duplicate samples for only one-fourth of the samples of plasma because three times as much sample was required as had been reported by Sobel and Snow. These duplicates, however, agreed within 2.46 percent.

The results of these determinations are presented in Table 30. The plasma vitamin A levels were not greatly affected by the vitamin A level of the diet. On the high vitamin A diet, plasma levels were comparable to those reported by Taylor et al. (1947); on the low vitamin A diets they were three to four times as high as on similar diets in Taylor's report. However, Taylor encountered more variability between hens than was found in the present studies.

The liver samples exhibited more drastic changes between diets than did plasma samples. Ranges in the liver samples were similar to those obtained by Harms et al. (1956) for chicks.

Birds on the vitamin A-deficient diet (E) for 63 days, as well as those on a supposedly adequate diet (D), had almost completely depleted the liver storage of vitamin A. Birds which had previously received a diet containing a very high level of vitamin A, had livers with much higher levels of vitamin A than did other groups.

Regression analysis of these data (vitamin A content of liver versus the incidence of blood spots produced or egg production) resulted in a correlation coefficient of less than .001. This extremely small degree of association suggests that blood spots are not caused by an inability of the hen to utilize vitamin A. Diets deficient in vitamin A cause a rapid depletion of liver storage of this vitamin which, in

TABLE 30

VITAMIN A CONTENT OF PLASMA AND LIVER SAMPLES FROM 44 WEEK-OLD HENS SUMMARIZED BY TREATMENT

Experimental diets		Pre-experimental vitamin A units per pound		
Diet	Vit. A levels[1]	"Low blood spot hens 8067 units	"High blood spot" hens 8067 units	12067 units
		(A) Plasma samples		
		mcg/ml	mcg/ml	mcg/ml
C	8067 units	3.49 ± 0.13	3.10 ± 0.07	3.16 ± 0.10
D	2156 units	2.75 ± 0.06	2.69 ± 0.07	3.19 ± 0.09
E	0 units	2.56 ± 0.06	2.63 ± 0.06	3.11 ± 0.11
		(B) Liver samples		
		mcg/gm	mcg/gm	mcg/mg
C	8067 units	30.09 ± 3.85	32.25 ± 4.72	147.75 ± 15.32
D	2156 units	1.74 ± 0.25	2.83 ± 0.32	54.68 ± 7.34
E	0 units	0.20 ± 0.002	1.20 ± 0.10	12.53 ± 2.56

1 USP units per pound, calculated on basis of corn, alfalfa, A and D oil, and a vitamin A palmitate supplement.

turn, causes blood spots. It is also suggested that under practical conditions the use of a diet that provides no margins of safety (diet D, on a calculated basis) may, due to rapid destruction of this vitamin during storage of feeds, actually be deficient and may cause an increased incidence of blood spots.

The variations in egg production presented in Table 28 were analyzed and differences found not to be significant (Table 31).

A summary of the results obtained on diets containing different levels of vitamin A and vitamin B_{12} is given in Table 32. Diets containing high levels (four to six times requirements) of vitamin A had a tendency, which was non-significant, to produce fewer eggs with blood spots. When the level of vitamin A was reduced to the National Research Council requirement level, or practically zero, caged birds - in two tests - showed a significant increase in incidence of blood spots; groups which had previously been on a diet containing six times the required level did not.

Birds on diets containing very high levels of vitamin B_{12} (30 and 60 micrograms per pound) showed a significantly increased occurrence of blood spots (Table 32 - Section B). This effect of very high level of vitamin B_{12}, however, appeared to be counteracted somewhat by very high levels of vitamin A (14,915 and 33,451 USP units per pound).

TABLE 31

ANALYSIS OF VARIANCE OF EGG PRODUCTION AS AFFECTED BY HIGH, NORMAL AND LOW DIETARY LEVELS OF VITAMIN A

Source of variation	Degrees of freedom	Mean square	F	F .05
Between	5	145.6	1.11	2.36
Within	65	131.1		

TABLE 32

SUMMARY OF THE EFFECT OF LEVELS OF VITAMIN A ON THE CHANGE IN INCIDENCE OF BLOOD SPOTS FOR FLOOR AND CAGED MANAGED BIRDS
(Vitamin A levels in USP units per pound of diet)

Pre-experimental (3 wk.)	First experiment Level	9 weeks Change	Second experiment Level	9 weeks Change
A: Caged layers				
8067	8067	-	8067	+
8067	8067	-	2156	+*
8067	8067	-	0	+**
8067	12067	-	8067	-
8067	12067	-	2156	-
8067	12067	-	0	+**
B: Floor layers				
8451	8451	+	8451	-
8451	14915[1]	-	9000	-**
8451	33451[1]	+*	4500	-*
8451	14951[2]	+	2250	+
8451	8451[2]	+**	0	-*

1 In addition contained 30 mcg of vitamin B_{12}
2 In addition contained 60 mcg of vitamin B_{12}
* Significant change $P < .05$
** Significant change $P < .01$

DISCUSSION

Intrafollicular hemorrhages occurring prior to ovulation have been established as the primary cause of blood spots in chicken eggs (Nalbandov and Card, 1944). It is possible that small blood vessels or capillaries rupture under the pressure of the rapidly developing yolk. If individual hens differ in the fragility of their capillaries in the highly vascularized, follicular membrane, this might account for the fact that some hens are more likely to lay blood spots than others.

Vitamin P reduces capillary fragility in certain human diseases (Armentano et al., 1956) but hens producing blood spots have not responded to this vitamin (Nalbandov and Card, 1944). Increased capillary fragility may also occur in hypertensive patients (Slovinsky, 1948). Could, therefore, hypertension and the incidence of blood spotting in chicken eggs be associated?

Increasing the level of salt (NaCl and/or KCl) in the diet did not significantly affect the incidence of blood spots - and was without effect on egg production or mortality. The levels employed, however, were less than the five percent shown necessary to produce hypertension (Krakower and Heino, 1947).

Atherosclerosis occurs naturally in chickens (Dauber, 1944; Patterson et al., 1949) and it can also be produced experimentally by feeding high levels of cholesterol (Dauber and Katz, 1943). Sclerotic lesions reduce the distensibility of arterial walls and are associated with an increase in blood pressure (Weiss et al., 1957). Consequently, a high level of cholesterol might possibly influence the incidence of blood spotting. However, feeding a substantial level of cholesterol did not do this.

A high level of fat has also been associated with the development of cholesterol-induced atherosclerosis in chickens. It was of interest, therefore, to find out if this would cause an increased incidence of blood spots. However, the addition of five percent of cottonseed oil to a low-cholesterol diet significantly reduced the incidence. Berg and Bearse (1958) showed an increase in blood spots in birds fed high energy diets but the level or type of fat used was not stated. It must have been above five percent to contain 1,450 calories of metabolizable energy per pound. If these workers used an animal fat, the difference in results could be explained on the basis of the work of Ahrens _et al_., (1954) who showed that vegetable fats decreased plasma cholesterol whereas animal fats caused an increase.

Researchers have shown that insufficiencies of certain lipotrophic factors play a role in atherosclerosis (Peters and VanSlyke, 1946 and Best and Lucas, 1943). High levels of i-inositol or betaine-HCl, however, did not cause a reduction in incidence of blood spots. Stamler _et al_., (1950), did not obtain any effect from feeding high levels of choline or inositol on experimentally induced atherosclerosis but Patterson _et al_., (1942) stated that cholesterol inhibited the lipotrophic activity of choline in formation of phospholipids. In the studies reported here, as well as those by Stamler, the basal diets supplied the required level of choline.

The synthesis of phospholipids is affected by vitamin D. Howland and Kramer (1921) reported that during the early stage of vitamin D deficiency, blood phosphorus and lipid levels were lowered in children. If lack of vitamin D can cause abnormal lipid metabolism, it might have an affect on vascular nutrition. In the present tests, vitamin D was removed from the ration or supplied in normal or high amounts. Deficient groups showed a non-significantly increased incidence of blood spots whereas those having a normal or high level of vitamin D showed little or no change. That high dietary levels of vitamin D did not reduce blood spot incidence had previously been reported by Nalbandov and Card (1944), and Berg and Bearse (1958).

Responses to unidentified growth factors (UGF) are associated to a substantial extent with the ash of certain feedstuffs (Scott, 1958). Since enzyme systems are greatly affected by certain minerals, (Dixon and Webb, 1958) and practical rations may be low in UGF, it was felt desirable to determine their effect on blood spots. Diets containing UGF at rather high levels, however, did not cause a consistent change in incidence. The basal diet contained fish meal and dried whey which may have satisfied the chicken's requirement in this regard, if they are needed in the prevention of blood spots.

The rate of recovery of rats from wounds was greatly reduced if they were fed high-salt diets deficient in potassium (Schwartz, et al., 1956), or when they were fed protein-deficient diets (Udupa et al., 1956). In view of this and the fact that vascular damage may occur more frequently when the birds are subjected to these stress conditions, it was wondered if similar treatments might have an effect on blood spots.

Large imbalances of Na and K in the diet caused chicks to grow slower and to have increased mortality (Burns et al., 1953). In the present experiments, however, high levels of NaCl with minimal levels of KCl or high levels of KCl with minimal levels of NaCl did not show any consistent trend in blood spot incidence. The levels employed were not as widespread as those employed by Schwartz, et al. (1953), or Burns et al. (1953), but were within ranges that might be encountered in practice when birds are fed mash and grain free-choice. However, it would appear that laying hens can tolerate rather wide variations in dietary Na and K levels, if neither are actually deficient in the diet (Burns, 1952), without influencing blood spots.

In addition to investigating the level of protein with respect to the blood spot picture, arsanilic acid was included because several workers (West and Hill, 1955; Carlson, 1957; Pope and Schaible, 1958) have shown that this compound reduced protein requirements and is associated with blood formation (Frost, 1953). However, birds fed low- or normal-protein diets, with or without arsanilic acid, did not exhibit significant changes in the incidence of blood spots. The implications involved here are that the modest changes in protein intake which might occur with birds fed by the mash and grain system would not account for the appearance of blood spots.

Birds fed on a free-choice mash-grain system of feeding exhibit wide variations in the amounts of each they consume. This even becomes a greater problem on a free-choice concentrate and grain system of feeding. Under both conditions, on a flock basis, the birds tend to consume less mash or concentrate than is needed to have a well balanced diet. What would happen if they consumed more mash than grain ? Their intake would be higher in vitamin B_{12} as well as protein and other critical nutrients. Would birds forced to eat high levels of vitamin B_{12} lay eggs containing fewer blood spots ? In these studies birds on diets containing very high levels (30 and 60 micrograms per pound) of vitamin B_{12} showed a significant increase.

Bearse et al., (1955) fed diets containing ten micrograms of vitamin B_{12} per pound and obtained a decrease in blood spots. One might suspect the very high levels used might be toxic. Brink et al. (1952)gave mice intraperitoneal dosages of 1600 milligrams per kilogram of body weight and no toxic symptoms were observed. Whether the carrier, itself, was responsible for the vitamin B_{12} effects in the present experiment is unknown. Numerous reports have been made concerning side effects resulting from the use of impure preparations of vitamin B_{12} from fermentation by-products (Bedford, 1952). Although an explanation for these results is not apparent, high levels of vitamin A (4, 5, and 11 times requirement) almost completely prevented the deleterious effects of the high levels of vitamin B_{12} and/or its carrier. This might indicate that these two vitamins are interrelated in some way and this needs further investigation.

Vitamin A is known to be necessary to retain a healthy condition of epithelial tissues (Follis, 1948). In field tests, the deficiency of vitamin A has been claimed to increase the incidence of blood spots in eggs (Bearse, 1955). Would birds which produce eggs with blood spots have a lowered metabolic efficiency in utilization and/or mobilization of vitamin A ? Present studies showed that birds fed diets containing high levels (4 to 6 times requirement) of vitamin A had a tendency, which was non-significant, to produce fewer eggs with blood spots (Table 29, Section A). When the level of vitamin A was reduced to NRC requirements or practically zero, caged birds in two tests significantly increased the incidence of blood spots; one group, which had previously been on a diet containing six times the required level, did not. A previously high dietary level, therefore, appears to modify the incidence of blood spots in a subsequent period if the vitamin A reduction in the diet is not too drastic. Thus, vitamin A at levels far in excess of the requirement did not significantly affect the incidence of blood spots but birds on a low or deficient level of vitamin A showed a significantly increased incidence of blood spots.

Bearse (1955) stated that a deficiency of vitamin A caused a high incidence of blood spots and that a level of 2270 units reduced this. Bearse also found that levels of 3632 and 9980 caused a slight, non-significant decrease. This worker used a different approach than the author and based his opinion on group averages rather than changes for individual hens. However, both experiments indicate that very high levels of vitamin A tend to reduce the incidence of blood spots and deficient diets cause a significant increase. Studies of the vitamin A content of blood and liver samples do not explain the lack of relationship between dietary vitamin A and incidence of blood spots.

High levels of vitamin A with other fat-soluble vitamins decreased the incidence of blood spots non-significantly. This suggests that the effect of these combinations was due to vitamin A.

Since vitamin E functions as an antioxidant (Ames, 1956), high levels might be expected to help lower the incidence of blood spots by protecting vitamin A. High vitamin E diets, however, did not have this effect. The form of vitamin E used in these studies, however, was the acetate, which is not effective as an antioxidant until it reaches the stomach and is released (Ames, 1956). Consequently, it would afford no protection in the feed against oxidation from the time of mixing until consumption.

CONCLUSIONS

Eight experiments were conducted using 456 Single Comb White Leghorn pullets (strain-cross and hybrid) to determine the effect of certain nutrients and other materials on the incidence of blood spots in chicken eggs. The incidence of blood spots of individual birds within a group, before and after a change in diet, was the criterion used to evaluate the dietary treatments employed.

The following dietary changes did not affect the incidence of blood spots:

Higher than normal levels of

Vitamins A, D, E and K
Cholesterol
i-inositol
betaine-HCl

Lower than normal levels of

sodium
chlorine
protein
vitamin D

Abnormal balances of

sodium and potassium
sodium and chlorine

Addition of

arsanilic acid
corn fermentation solubles
Vigofac

The incidence of blood spots was increased significantly by the following:

Very high levels of vitamin B_{12}
Diets containing 2156 or 0 USP units of vitamin A per pound

The effect of very high levels of vitamin B_{12} was counteracted with a very high level of vitamin A.

A consistent but non-significant reduction in the incidence of blood spots was obtained with diets containing very high levels of vitamin A.

A significant reduction in the incidence of blood spots was obtained on a diet containing five percent refined cottonseed oil.

Literature Cited

Ahrens, E. H., D. H. Blankenhorn, and T. T. Tasltas, 1954. Effect on human lipids of substituting plant fat for animal fat in diet. Proc. Soc. Exptl. Biol. and Med. 86:872-884.

Ames, S. R., 1956. Role of vitamin E (alpha-tocopherol) in poultry nutrition and diseases. Poultry Sci. 35:145-159.

Aristotle, Transl. by A. Platt, 1910. De generatione animalium III,2, 752 C 1-100. As quoted by Nalbandov, A. V., and L. E. Card, 1944. The problem of blood clots and meat spots in chicken eggs. Poultry Sci. 23:170-180.

Armentano, L., A. Bentsath, T. Beres, Ste. Rusznyak and A. Szent-Gyorgi, 1936. Effect of substances of the flavone groups on capillary permeability. Vitamin P. Deutsch. Med. Wachenschr., 62:1325-1328. Nutrition Abs. and Reviews, 1936. 37:667.

Baten, W. D., and E. W. Henderson, 1958. Method of weighing hatching data in statistical analysis. Poultry Sci. 37:781-785.

Bearse, G. E., 1955. Effect of alfalfa, vitamins on egg blood spot incidence. Flour and Feed. 56:26-27.

Bearse, G. E., C. F. McClary and H. C. Saxen, 1953. Bloodspot incidence and the vitamin A level of the diet. Poultry Sci. 32:888.

Bedford, P. D., 1952. Side-effects of a preparation of vitamin B_{12}. Brit. M. J. 1:690-691.

Benjamin, E. W., and H. C. Pierce, 1937. Marketing poultry products. Third edition, John Wiley and Sons, Incorporated, New York.

Bentsath, A., Ste. Ruszmayak and A. Szine-Gyorgyi, 1936. Vitamin nature of flavones. Nature. 138:798.

Berg, L. R., and G. E. Bearse, 1958. The fat soluble vitamins and blood spot incidence. Poultry Sci. 37:1184-1185.

Best, C. H. and C. C. Lucas, 1943. Choline- chemistry and significance as a dietary factor. Vitamins and Hormones. 1:1-51.

Bird, H. R., 1952. Arsonic counpounds in feeds. Feed Age. 2:29.

Bird, H. R., H. J. Almquist, W. S. Cravens, F. W. Hill and J. McGinnis, 1954. Nutrient requirements for poultry. National Academy of Science-National Research Council, Publ. 301:1-27.

Brink, N. G., F. A. Kuehl, Jr. and K. Folkers, 1950. Vitamin B_{12}: the identification of vitamin B_{12} as a cyano-cobalt coordination complex. Science 112:354.

Burmester, B. R. and L. E. Card, 1938. On the nature of "meat spots" in eggs. Poultry Sci. 17:235-239.

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

Burns, C. H., W. W. Cravens and P. H. Phillips, 1952. The requirement of breeding hens for sodium chloride. Poultry Sci. 31:302-306.

Camp, A. A., H. T. Cartrite, B. L. Reid, J. H. Quisenberry and J. R. Couch, 1957. Corn steepwater solubles as a source of unknown growth factor(s) for growing chicks. Poultry Sci. 36:1354-1359.

Carlson, C. W., 1957. Some effects of arsanilic acid and/or penicillin upon egg production. Poultry Sci. 36:1070-1075.

Carver, J. S. and W. Henderson, 1948. The effect of rutin and ascorbic acid and of alfalfa on blood and meat spots in chicken eggs. Poultry Sci. 27:656.

Dam, H., and J. Glavinal, 1938. Alimentary exudative diathesis, Nature. 142:1077-1078.

Dauber, D. V., and L. N. Katz, 1943. Experimental atherosclerosis in the chick. Arch. Path. 36:473-492.

Dauber, D. V., 1944. Spontaneous atherosclerosis in chickens. Arch. Path. 38:46-51.

Dawson, L. E., 1953. Increase income by reduction of blood and meat spots in chicken eggs. Poultry Processing and Marketing. 59:11,16.

Dawson, L. E., 1955. Bloodspots - who should shoulder the responsibility. Poultry Processing and Marketing. 61:18-19,22.

Dawson, L. E., and H. R. Richardson, 1952. Blood and meat spots in chicken eggs. Mich. Agr. Expt. Sta. Quart. Bul. 35:110-119.

Denton, Charles A., 1947. Observations on the incidence and characteristics of blood spots in hen's eggs. Poultry Sci. 26:272-276.

Donavan, G. A., W. K. Warden and W. M. Reynolds, 1958. A comparison of unidentified growth factor sources for turkeys and influence of calorie-protein ratio on response. Poultry Sci. 37:422-428.

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

Fabricius 1600, by Adelmann, H. B., 1942. The embryological treatise of Hieronymous Fabricius of Aquapendente. Cornell Univ. Press, Ithaca, New York.

Farnsworth, G. M., and A. W. Nordskog, 1955. Estimation of genetic parameters influencing blood spots and other economic traits of the fowl. Poultry Sci. 34:1142.

Follis, R. H., Jr., 1948. The pathology of nutritional diseases; physiological and morphological changes which result from deficiencies of elements, amino acids, vitamins and fatty acids. Charles C. Thomas, Springfield, Ill.

Fowler, T., 1786. Medicinal report of the effect of arsenic in the cure of Agues, etc. Printed for J. Johnson and Wm. Brown, London.

Frost, D. V., 1953. Considerations on the safety of arsanilic acid for use in poultry feed. Poultry Sci. 32:217-227.

Gallup, W. D. and J. A. Hoefer, 1946. Determination of vitamin A in liver. Ind. and Eng. Chem. Annal. Ed. 18:288-298.

Gies, T., 1877. Experimentalle utersuchungen uber den einfluss des arsens auf den organismus. Arch. F. Exp. Path. U. Pharm. 8:175-197.

Harms, R. H., B. L. Reid and J. R. Couch, 1956. Further studies on a factor obtained from condensed fish solubles which enhances vitamin A storage in chick livers. Poultry Sci. 35:1224-1258.

Helbacka, N. V. and M. H. Swanson, 1958. Studies on blood and meat spots in hen's egg. 1. The fluorescent property of meat spots. Poultry Sci. 37:869-876.

Helbacka, N. V. and M. H. Swanson, 1958. Studies on blood and meat spots in hen's egg. 2. Some chemical and histological characteristics of blood and meat spots. Poultry Sci. 37:877-885.

Hill, C. H., and H. W. Garren, 1958. Effect of dietary protein levels on susceptibility of chicks to fowl typhoid. Federation Proc. 17:479.

Howland, J., and B. Kramer, 1921. Calcium and phosphorus in serum in relation to rickets. Am. J. Diseases Children 22:105-119.

Jeffrey, F. P., 1945. Blood and meat spots in chicken eggs. Poultry Sci. 24:363-374.

Jeffrey, F. P., and J. Pino, 1943. The effects of heredity and of certain environmental factors on the incidence of blood spots in chicken eggs. Poultry Sci. 22:230-234.

Katz, L. N. and J. Stamler, 1953. Experimental Atherosclerosis. Charles C. Thomas, Springfield, Ill.

King, S. C. and G. O. Hall, 1955. Egg quality studies at the New York random sample test. Poultry Sci. 34:799-809.

Krakower, C. A. and H. E. Heino, 1947. Relationship of growth and nutrition to cardiorenal changes induced in birds by high salt intake. Arch. Path. 44:143-162.

Lenel, R., L. N. Katz and S. Rodbard, 1948. Arterial hypertension in the chicken. Am. J. Physiol. 152:557-562.

Lerner, I. M. and W. R. Smith, 1942. Effect of season and heredity on the incidence of bloodspots. Poultry Sci. 21:473.

Lerner, I. M. and L. W. Taylor, 1947. Seasonal and daily fluctuations in the incidence of blood spots.. Poultry Sci. 26:662-667.

Lerner, I. M., L. W. Taylor and Dorothy C. Lowry, 1951. Selection for increased incidence of blood spots in white leghorns. Poultry Sci. 30:748-757.

Libby, D. A., R. J. Evans, S. L. Bandemer and P. J. Schaible, 1955. Effect of long-time feeding of certain arsonic acids to chickens. Poultry Sci. 34:972-980.

McHenery, E. W. and M. L. Cornett, 1944. The role of vitamins in the anabolism of fats. Vitamins and Hormones. 2:1-24.

Merritt, E. B., 1950. Studies on the origin and nature of meat spots in eggs. Unpublished Master's thesis, Univ. of Massachusetts, Amherst.

Nalbandov, A. V. and L. E. Card, 1944. The problem of blood clots and meat spots in chicken eggs. Poultry Sci. 23:170-180.

Page, I. H., F. J. Stare, A. C. Corcuran, H. Pollach and C. F. Wilkinson, Jr., 1957. Atherosclerosis and the fat content of the diet. Circulation, N.Y. 16:163-178.

Paterson, J. C., C. A. Mitchell and A. C. Wallace, 1949. Experimental coronary sclerosis. II. The role of infection in coronary sclerosis of cockerels. Arch. Path. 47:335-339.

Peters, J. P. and D. D. VanSlyke, 1946. Quantitative Chemistry; Interpretations, 2nd ed. Williams and Wilkins, Baltimore, Md.

Pope, C. W. and P. J. Schaible, 1958. Effect of arsanilic acid in low- and normal-protein mashes upon egg production. Poultry Sci. 37:1234.

Pope, C. W. and P. J. Schaible, 1959. Effect of arsanilic acid in low- and normal-protein mashes upon egg production. Poultry Sci. 38: In press.

Quinn, J. P. and A. B. Godfrey, 1940. Inheritance and variation of blood spots in chicken eggs. Poultry Sci. 19:359-360.

Quinn, J. P. and A. B. Godfrey, 1948. Breeding for high and low incidence of blood spots in eggs of Rhode Island Reds. Poultry Sci. 27:680.

Sauter, E. A., W. J. Stadelman and J. S. Carver, 1952. Factors affecting the incidence of blood spots and their detection in hen's eggs. Poultry Sci. 31:1042-1049.

Schwartz, R., J. Cohen and W. M. Wallace, 1953. Tissue electrolyte changes of the whole body, muscles, erythrocytes and plasma of rats on a potassium deficient diet. Am. J. Physiology. 172:1-6.

Scott, H. M., E. Jungherr and L. D. Matterson, 1944. Possible role of potassium in pullet disease. Proc. Soc. Exptl. Biology and Med. 57:7-10.

Scott, M. L., 1957. Corn distillers dried solubles as a source of unidentified nutrients required by chickens and turkeys. Distillers Feed Conference, Proc. of. 12:61-70.

Sharma, G. P., 1949. Note on an abnormal intrafollicular ovum. Poultry Sci. 28:310-314.

Slovinsky, T., 1948. La fragilidad capilar in las hipertensas. Effecto del rutin y las vitamin C y K. Rev. Argentina Cardiol. 15:274-285.

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

Sobel, A. E. and S. D. Snow, 1947. The estimation of serium vitamin A with activated glycerol dichlorohydrin. J. Biol. Chem. 171:617-632.

Stiles, P. G., 1958. Studies on the cause and incidence of blood spots in chicken eggs. Unpublished Doctorate thesis, Michigan State Univ., East Lansing.

Taylor, M. W., J. R. Stern and W. C. Russell, 1947. The provitamin A requirement of hens. Poultry Sci. 26:243-254.

Udupa, K. N., J. F. Woessner and J. E. Dumphy, 1956. The effect of methionine on the production of mucopolysaccharides and collagen in healing wounds of protein-depleted animals. Surg. Gynic. Obstet. 102:639-645.

Weiss, H. S. 1950. The effect of neuromimelic drugs on egg formation. Unpublished Doctorate thesis, Rutgers Univ., New Brunswick, New Jersey.

Weiss, H. S., R. K. Ringer and P. D. Sturkie, 1957. Relationship amoung plasma cholesterol, artic cholesterol and blood pressure in the hen. Fed. Proc. 16:137.

West, J. W., and J. E. Hill, 1955. Protein requirement of broilers as influenced by antibiotics. Poultry Sci. 34:628-634.