THE EFFECT OF CERTAIN DIETARY INGREDIENTS UPON THE INCIDENCE OF BLOODSPOTS IN CHICKEN EGGS

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ABSTRACT

THE EFFECT OF CERTAIN DIETARY INGREDIENTS UPON THE INCIDENCE OF BLOODSPOTS IN CHICKEN EGGS

by James Britton Ward

Experiments were conducted with three strains of Single Comb White Leghorn hens to study the effects of certain dietary ingredients on the incidence of bloodspots -- particularly those concerned with internal hemorrhaging. The number and size of bloodspots in eggs on an individual bird basis occurring before, during and after feeding of the experimental diets were used as criteria to evaluate the effects of the dietary treatments.

Additions of vitamin B_{12}, selenium, crude cottonseed oil, crude corn oil, a refined soybean-corn oil blend, beta-aminopropionitrile fumarate (BAPN), vitamin K (menadione sodium bisulfite), reserpine (an antihypertensive agent), and combinations of reserpine with sulfaquinoxaline, sulfaquinoxaline with soybean-corn oil blend, vitamin K with soybean-corn oil blend and dehydrated alfalfa leaf meal plus animal fat were without influence on number or size of bloodspots produced. Differences in bloodspot incidence between strains of birds were noted.

The percentage of red blood cells (hematocrit value) was different for strains, periods and treatment while prothrombin times (blood clotting potential) were not different for strains or treatments. Prothrombin times were not determined for each period.

Hematocrit values were significantly depressed by a high level of cobalt while a low level of cobalt caused a non-significant increase in

hematocrit values. Values determined in July were lower than those in March or May.

The highest level of selenium (5 ppm), reserpine (2mg/lb) plus sulfaquinoxaline (0.125%) and soybean-corn oil blend plus sulfaquinoxaline (0.1%) caused a significant depression in egg production. The highest level of vitamin B_{12} resulted in a non-significant increase in production as did the addition of 1 ppm of selenium.

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By

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INTRODUCTION

From one-half to three percent of all eggs entering primary market channels are rejected because of bloodspots. Another five to seven percent of the eggs actually contain bloodspots which are too small to be detected by the candling process and thus are sold to the consumer. Bloodspots cause a substantial loss to poultrymen but the exact amount is difficult to estimate because of the difficulty in ascertaining what effect they may have on the consumer.

It has been shown that the heritability of bloodspot incidence is quite high. However, while some strains of birds have a very low incidence, it has been virtually impossible to eliminate bloodspots completely by selection and breeding.

For the purposes of experimentation, it would be desirable to find some means of increasing the incidence of bloodspots. From a practical standpoint, of course, it would be desirable to find some means of reducing the incidence of bloodspots. With these thoughts in mind the hypothesis was developed that perhaps bloodspot incidence could be influenced through nutrition by means of including or deleting certain dietary ingredients.

The studies reported herein involved dietary supplementation with various levels of vitamin B_{12}, selenium, vegetable and animal fats, vitamin K, sulfaquinoxaline, a tranquilizer, and beta-aminopropionitrile fumarate (BAPN). Of particular interest were treatments affecting hemorrhaging and blood clotting.

REVIEW OF LITERATURE

Nalbandov and Card (1944) reasoned that bloodspots were the result of intrafollicular hemorrhaging prior to ovulation and that hemorrhaging may have occurred several days before the release of the yolk. Later, Stiles et al. (1958) injected birds with radioactive phosphorus (P_{32}) 3 1/2 hours after ovulation and could detect no radioactivity in a bloodspot present at oviposition. When the radioactive phosphorus was injected nine hours prior to ovulation, radioactivity was detected in a bloodspot present in the egg at oviposition. These workers concluded that the formation of the bloodspot occurred sometime within the nine-hour period prior to ovulation.

Quinn and Godfrey (1940) reported that there were breed and family differences in bloodspot incidence. The heritability of bloodspots was estimated to be 0.32 by Farnsworth and Nordskog (1955), who based their estimation on a sample of 15 eggs per hen. Lerner et al. (1959) increased the incidence of bloodspots from between 0.5 and 1.3 up to 23 percent through selection. The heritability of bloodspotting, expressed as percent of all eggs laid which contained bloodspots, was found to be 0.5.

The effect of the absence or presence of certain dietary ingredients linked with capillary fragility, general subcutaneous bleeding and aortic rupture on the incidence of bloodspots has been investigated. A deficiency of vitamin P, a crude mixture of glycosides of eriodictin and herperidin, increases capillary fragility and permeability (Titus, 1955). Rutin, a crystalline glycoside of quercitin, effective in mending capillary fragility and preventing petechial bleeding in humans, has been studied with respect to bloodspot incidence as has vitamin C which causes

a decrease in capillary fragility in some cases but not in others (Tey, cited by Houssay, 1955). Carver and Henderson (1948) investigated the effect of rutin and vitamin C in the diet of laying hens but found no reduction in the incidence of bloodspots.

According to Yacowitz et al. (1955), 0.1 percent sulfaquinoxaline caused hemorrhaging at four to five weeks of age and decreased hematocrit values at two to three weeks of age. Cuckler and Ott (1955) reported that a level of at least 0.4 percent sulfaquinoxaline in the diet of broilers was required to produce a prolonged increase in blood clotting time and even this gave only a slight increase in prothrombin time. Anderson et al. (1954) stated that broilers developed hemorrhages when raised on a wire-floored battery and fed a simplified corn-soybean oil meal ration. This hemorrhagic condition could be overcome by the addition of vitamin K. Data presented by Hare et al. (1953) indicated that birds fed a corn-soybean oil meal ration from one to six weeks of age developed a condition similar to the so-called hemorrhagic disease. Balloun and Johnson (1952) increased blood clotting time by feeding underheated soybean oil meal to chicks. Additions of vitamin K did not restore blood clotting time to normal.

When Stephens and Tugwell (1960) fed a "low" vitamin K diet to chicks, blood clotting time was increased. Blood clotting time was decreased when 2.5 percent autoclaved and dried feces or three percent alfalfa leaf meal was added to the ration. Menhaden fish meal added to the diet did not influence blood clotting time. However, dicumarol in the ration of chicks increased blood clotting time (Harms and Tarver, 1957).

By feeding .036 percent beta-aminopropionitrile fumarate (BAPN),

Roy and Bird (1959) produced leg deformities and aortic rupture in chicks prior to six weeks of age. When Barnett and Morgan (1959) fed BAPN at .06 percent in both low and high fat diets, incidence of internal hemorrhaging was 9.5 percent and 15 percent, respectively. Waibel and Pomeroy (1958) produced aortic rupture in turkeys by feeding .02 percent BAPN. Vitamin K added to the diet did not consistently reduce BAPN-induced hemorrhaging (Barnett et al., 1958).

Card and Nalbandov (1944) reported that allowing birds to run on range reduced bloodspot incidence but that fresh-cut grass fed inside the poultry house was not as effective in reducing the incidence. However, Denton (1947) found no effect from the feeding of green grass on the incidence of bloodspots. Nalbandov and Card (1947) found no influence from feeding defatted wheat germ, wheat germ oil, liver meal, condensed fish solubles, yeast, a "mixture of trace minerals" or folic acid on bloodspot occurrence.

According to Sauter et al. (1952) ten percent of alfalfa leaf meal in the ration lowered the incidence of bloodspots. By feeding hens vitamin A at levels of 200, 300, and 400 IU per hundred grams of diet, Bearse et al. (1953) found that a decrease in bloodspots occurred as the vitamin A level increased. Later, Bearse and Berg (1958) reported that vitamin A levels of 3,000 and 6,000 IU/lb. lessened the bloodspot problem. The incidence of bloodspots was greater on high energy diets than on low energy diets. The authors attributed this higher incidence to a twenty percent reduction in feed intake with a consequent reduction in vitamin A intake for birds on the high energy diets. Bearse et al. (1960) found that vitamin A levels of 1200 to 1600 IU/lb. produced a minimum of bloodspots and that no further reduction occurred from in-

creasing the vitamin A level up to 15,000 IU/lb. Munro (1952) reported that the percentage of bloodspots, as determined by candling, was consistently lower on a low energy ration as compared to a high energy ration (1.77 vs. 2.91 percent).

Using various levels of sodium chloride, potassium chloride, sodium carbonate and their combinations, Pope et al. (1961) found no influence on the incidence of bloodspots. Vitamins E and K, corn fermentation products and Vigofac were also without influence while "low" levels of vitamin A caused an increase in bloodspot incidence.

When Pope (1959) fed high levels (ten to twenty times the normal amount) of vitamin B_{12}, there was an increase in the number of bloodspots. When these high levels were fed with vitamin A, the adverse effect of vitamin B_{12} was reduced somewhat. Depleting hens of vitamin B_{12} or supplementing B_{12} depleted birds had no effect on the incidence of bloodspots (Bearse, 1961). Three grams of vitamin K and two pounds of methionine per ton in the ration of caged layers did not influence the incidence of bloodspots.

Erythrocytosis (abnormal production of red blood cells) can be induced by the administration of "excessive" doses of vitamin B_{12} or other cobalt compounds with a resultant increase in peripheral resistance which may in turn lead to hypertension (Kugelmass, 1959). Weiss (1958) reported that incidence of bloodspotting was higher in a high blood pressure line of birds than in a low blood pressure line, but not significantly so. Speckmann and Ringer (1961) reduced the blood pressure in turkeys by feeding reserpine. Sturkie (1959) reported that reserpine causes hypotensive changes in chickens.

EXPERIMENTAL PRODECURE AND RESULTS

General Procedure

The hens were housed in individual double-tiered, offset-type cages. Cages were arranged in groups of eight and feed and water were provided ad libitum. Water troughs extended the full lenght of each row of cages and were cleaned daily. Manure was removed once each week with an automatic pit cleaner.

Eggs were collected daily and examined twice weekly on the same days of each week. All eggs were broken out on a glass plate with mirrors both underneath and behind. This permitted full observation of the egg for bloodspots. Bloodspots were recorded in one of three groups according to size: 1/16th inch (1.6 millimeters) or less in diameter; greater than 1/16th inch (1.6 millimeters) but less than 3/16th inch (4.8 millimeters) in diameter; 3/16th inch (4.8 millimeters) or greater in diameter. Bloodspot data from hens laying in only one of two periods being compared were not included.

The effect of treatment on bloodspot incidence was analyzed statistically on the basis of the number of birds influenced in a like manner. Treatments were group as follows: 1 through 6, 7 through 9 and 11 through 16. The groups will be referred to as Groups 1, 2 and 3 respectively. Treatment 10 was not considered in the analysis because the birds were not kept on the experimental diet the allotted time. The Chi-square method (Snedecor, 1956) was employed because it is designed to measure the deviation of frequency of sample numbers in different categories from the expected frequency. In this case, birds were placed in one of three categories based on change in bloodspot incidence for each bird between anv two periods.

For the analysis of egg production data hematocrit values and prothrombin times, the analysis of variance (Snedecor, 1956) was employed. Tests for significant differences between means were conducted according to the method of Duncan (1955).

Hematocrit values were determined by the micro technique as described by McGovern et al. (1955). Blood from the brachial vein in the wing was collected directly into capillary tubes. Centrifugation was carried out in a micro-capillary centrifuge and readings were made on a micro-capillary reader according to the method outlined by Jones (1956).

INTRODUCTION TO EXPERIMENTS A AND B

Conflicting reports with reference to the influence of vitamin B_{12} on bloodspot incidence exist in the literature. There are indications that excessively high levels of vitamin B_{12} increases the number of bloodspots, but this influence may be modified by factors such as various levels of vitamin A. Since vitamin B_{12} and cobalt have been reported to stimulate erythrocyte production, hematocrit values (percentage of red blood cells) were determined.

Pope (1959) obtained a slight decrease in bloodspot incidence by feeding crude cottonseed oil. On the basis of Pope's work the decision was made to compare the effect of vegetable oils and animal fat on the incidence of bloodspots in chicken eggs.

In recent years, vitamin K has received considerable attention in the popular press as being important in decreasing bloodspot incidence. In addition, claims are being made that the vitamin K currently being used is more stable. Although earlier experimental work had indicated no effect from vitamin K on bloodspot incidence, it seemed advisable to conduct new tests in light of the greater stability claims and reports of reduced bloodspot incidence as a result of feeding the more stable vitamin K. Beta-aminopropionitrile and sulfaquinoxaline, were included because of their association with internal hemorrhaging.

Since reserpine is well known as a hypotensive agent it was theorized that if there was a correlation between high blood pressure and bloodspot incidence as suggested by Weiss (1958) then reserpine should cause a reduction in incidence of bloodspots.

Experiment A: Procedure and Results

The effects of vitamin B_{12}, cobalt, selenium and fat or oil were studied with respect to bloodspot incidence. The experiment was divided into five intervals denoted as Periods 1 through 5. During Periods 1, 4 and 5 all birds were fed the same basal diet; during Periods 2 and 3, birds on treatments 1, 7 and 11 were on basal; all others were on experimental diets. Periods 2 and 4 were 14 days each in length and were considered "switch over" periods. Observations for bloodspots were made during these periods but were not included in the data.

Periods 1 and 5 were each 60 days in length for all treatments. Period 3 was 60 days in length for treatments 1 through 6 and 68 days in length for treatments 7 through 16. At the end of 60 days, all of the experimental diets for treatments 7 through 16 had not been consumed, thus Period 3 was extended for these treatments in order to utilize the experimental diets completely.

The calendar dates for this experiment were as follows:

	Group 1	Group 2	Group 3
Period 1	Dec. 29 to Feb. 26	Nov. 19 to Jan. 17	Same
Period 2	Feb. 27 to Mar. 11	Jan. 18 to Jan. 31	as
Period 3	Mar. 12 to May 10	Feb. 1 to Apr. 18	group
Period 4	May 11 to May 24	Apr. 9 to Apr. 22	2
Period 5	May 25 to July 23	Apr. 23 to June 21	

Three strains of birds were used. One had a history of "high" incidence (Strain A), another a history of "medium" incidence (Strain B) and the third a history of "low" incidence (Strain C). There were 24 birds per treatment in Group 1 and 16 birds per treatment in Groups 2 and 3. Group 1 contained birds from Strains A, B and C; Group 2 from

strains B and C; Group 3 from strain C only.

Group 1 was fed various levels of vitamin B_{12} or cobalt (the lowest level of cobalt was the equivalent of that found in 30 micrograms of vitamin B_{12}; the highest level of cobalt was the equivalent of that found in a commercial trace mineral mix). Group 2 was fed different levels of selenium and Group 3 different levels of vegetable oils and animal fat.

A total of 34,155 eggs were examined in this experiment and the data are summarized in Tables 3 through 12 and Appendix Tables 1 through 13.

Table 1. Composition of the Basal Ration Fed in Experiment A

Ingredient	Amount per Cwt.
Corn, yellow No. 2, ground	66.70 lbs.
Soybean oil meal, 44% protein	19.00 "
Meat and bone scrap, 50% protein	3.00 "
Fish meal (with solubles), 60% protein	1.25 "
Alfalfa leaf meal, dehyd., 20% protein	1.30 "
Fat, animal, No. 2 yellow grease	1.50 "
Dicalcium phosphate, feed grade	2.00 "
Limestone, ground	5.00 "
Salt, iodized	.25 "
Manganese sulfate, 70% feed grade	.025 "
Riboflavin suppl. (16 gm/lb)	1 gram
Vitamin A (10,000 IU/gm)	20 "
Vitamin D_3 (3,000 ICU/gm)	10 "
Total	100.00 + lbs.

Calculated analysis:

Crude protein	%	16.9	Vitamin B_{12}	(mcg/lb)	1.4
Crude fat	%	4.5	Riboflavin	(mg/lb)	1.3
Crude fiber	%	3.1	Niacin	(mg/lb)	10.9
Calcium	%	2.8	Pantothenic acid	(mg/lb)	2.9
Phosphorus	%	.8	Choline	(mg/lb)	406
Vitamin D (ICU/lb)		300	Energy	(Cal/lb)	945
Vitamin A (IU/lb)		3563			

Table 2. Changes in Basal Ration for Experiment A

Ingredients	Units	Diets 1	2	3	4	5	6
Vitamin B_{12}	mcg/lb	--	15	30	60	--	--
Cobalt (as Co CO_3)	mcg/lb	--	--	--	--	1.32	90

		Diets 7	8	9	10
Selenium (as Na_2 Se $O_3 \cdot 5H_2O$)	ppm	--	1	5	10

		Diets[1] 11	12	13	14	15	16
Animal fat	percent	--	3.5	--	--	--	- 1.5
Crude cottonseed oil	percent	--	--	5.0	--	--	--
Soybean-corn oil[2]	percent	--	--	--	5.0	--	--
Crude corn oil	percent	--	--	--	--	5.0	--

[1] Changes in the percentages of corn were made to compensate for additions or subtractions of fat or oil.

[2] A blend containing refined soybean oil and corn oil in a 4:1 ratio.

Table 3. Change in Bloodspot Incidence Between Periods 1 and 3 When Calculated on an Individual Bird Basis

Treatment	Average percent change	Percentage of birds increasing	Percentage of birds decreasing	Percentage of birds showing no change	Chi square values
1	- 2	43	57	0	2.15
2	+ 1	44	44	12	2.71
3	- 13	11	78	11	3.97
4	- 5	35	60	5	.11
5	- 10	27	68	5	.53
6	- 2	35	55	10	.42
Total					9.89
Chi-square value 10 degrees of freedom ($P < .01$) = 23.21					
7	+ 3	60	20	20	1.23
8	+ 1	42	50	8	1.59
9	+ 1	50	33	17	.02
Total					2.84
Chi-square value 4 degrees of freedom ($P < .01$) = 13.28					
11	+ 4	28	36	36	3.23
12	- 3	31	46	23	.26
13	- 3	25	56	19	.98
14	- 2	19	62	19	1.19
15	- 2	47	47	6	2.79
16	- 2	38	54	8	1.22
Total					9.67
Chi-square value 10 degrees of freedom ($P < .01$) = 23.21					

Table 4. Change in Bloodspot Incidence Between Periods 3 and 5 When Calculated on an Individual Bird Basis

Treatment	Average percent change	Percentage of birds increasing	Percentage of birds decreasing	Percentage of birds showing no change	Chi square values
1	+ 4	53	42	5	.49
2	- 7	31	62	6	7.45
3	+ 3	53	29	18	1.46
4	+ 8	62	38	0	1.86
5	+ 6	59	35	6	.32
6	+ 5	61	11	28	9.82
Total					21.40
Chi-square value 10 degrees of freedom ($P < .01$) = 23.21					
7	- 8	15	69	16	.41
8	- 2	33	67	0	2.36
9	- 4	20	50	30	2.11
Total					4.88
Chi-square value 4 degrees of freedom ($P < .01$) = 13.28					
11	- 1	17	58	25	1.83
12	- 1	33	59	8	.46
13	- 2	30	67	13	1.39
14	- 1	43	36	21	1.54
15	+ 1	36	50	14	.04
16	+ 3	50	42	8	1.72
Total					6.98
Chi-square value 10 degrees of freedom ($P < .01$) = 23.21					

Table 5. Percentage Egg Production for Periods 1 and 3

Treatment	Period 1	Period 3	Amount of change
	%	%	
1	64	51	- 13
2	62	54	- 8
3	59	59	0
4	58	67	+ 9
5	69	60	- 9
6	58	55	- 3
7	57	65	+ 8
8	59	75	+ 16
9	64	36	- 28
11	70	71	+ 1
12	69	69	0
13	79	68	- 11
14	72	69	- 3
15	63	60	- 3
16	68	69	+ 1

Table 6. Percentage Egg Production for Periods 3 and 5

Treatment	Period 3	Period 5	Amount of change
	%	%	
1	51	54	+ 3
2	54	66	+ 12
3	59	57	- 2
4	67	67	0
5	60	64	+ 4
6	55	48	- 7
7	65	68	+ 3
8	75	65	- 10
9	36	53	+ 17
11	71	53	- 18
12	69	51	- 18
13	68	72	+ 4
14	69	68	- 1
15	60	56	- 4
16	69	66	- 3

Table 7. Analysis of Variance of Egg Production Data for Birds in Group 1 for Periods 1, 3 and 5

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	5	387.8	1.89	3.06
Strains	2	1087.0	5.29	4.66
Periods	2	119.5	.58	4.66
T X P	10	215.7	1.05	2.46
T X S	10	286.6	1.31	2.46
P X S	4	347.8	1.70	3.36
T X P X S	20	220.0	1.07	1.61
Error	311	205.1		

Strains	A	B	C
Av. eggs/hen	32.2	37.2	38.0

Means not underscored by the same line are significantly different

Table 8. Analysis of Variance of Egg Production Data for Birds in Group 2 for Periods 1, 3 and 5

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	2	1018.5	5.19	4.82
Strains	1	1118.0	5.70	6.90
Periods	2	148.0	.75	4.82
T X P	4	836.7	4.27	3.51
T X S	2	517.5	2.64	4.82
P X S	2	542.5	2.77	4.82
T X P X S	4	165.2	.84	3.51
Error	103	196.1		

Since the T X P interaction was significant for this group, an analysis of variance was conducted on data of all periods individually in order to determine if treatments had a significant effect within each period. The analysis of variance tables for individual periods are in the Appendix.

Table 9. Analysis of Variance of Egg Production Data for Birds in Group 3 for Periods 1, 3 and 5

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	5	264.0	1.47	3.11
Periods	2	1413.0	7.87	4.71
T X P	10	211.1	1.17	2.41
Error	243	179.4		

Periods	5	1	3
Av eggs/hen	36.9	42.2	45.0

Table 10. Hematocrit Values for Birds in Group 1

Treatment	Period 1	Period 3	Period 5
1	28.4	28.5	26.7
2	28.1	29.7	26.8
3	28.4	29.4	26.8
4	29.4	28.6	26.7
5	27.1	28.1	28.0
6	29.8	27.3	26.5
Strains:			
A	27.2	27.6	25.4
B	29.0	28.8	27.4
C	29.1	29.1	27.5

Table 11. Analysis of Variance for Hematocrit Values for Birds in Group 1 for Periods 1, 3 and 5

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	5	3.50	.63	3.09
Periods	2	95.26	17.91	4.69
Strains	2	98.03	17.69	4.69
T X P	10	17.88	3.22	2.39
T X S	10	4.03	.72	2.39
P X S	4	5.33	.96	3.39
T X P X S	20	6.37	1.15	1.95
Error	298	5.54		

Periods	5	1	3
Hct. values	26.9	28.5	28.6
Strains	A	B	C
Hct. values	26.9	28.5	28.6

Means not underscored by the same line are significantly different ($P < .01$).

Since the T X P interaction was significant an analysis of variance was conducted on data of all periods individually in order to determine if treatments had a significant effect within each period. The analysis of variance tables for the individual periods are in the Appendix.

Table 12. Results of Grading of Eggs by a Commercial Grader

Grade	Number of eggs	Percent of total
A	317	88.2
B	23	6.3
C and Checks	12	3.3
Rejects (bloodspots)	8	2.2

Discussion of Experiment A:

For the control ration and treatments involving various levels of vitamin B_{12} (15, 30 and 60 micrograms per pound in Treatments 2, 3 and 4, respectively), and cobalt at the lowest level (treatment 5) there was a non-significant decrease in bloodspot incidence when expressed as percent of all eggs laid (Appendix Table 1). There was a slight increase in bloodspot incidence with the highest level of cobalt. When expressed as average percent change on an individual bird basis, the lowest level of vitamin B_{12} (treatment 2) showed a slight increase (Table 3). All other treatments showed a slight decrease. This is in agreement with the work of Bearse (1961), who found that including vitamin B12 in the diet of depleted birds or depleting birds of vitamin B_{12} had no influence on number of bloodspots.

Selenium at any of the levels fed was without influence on the number of bloodspots (treatments 7 - 9). Birds fed the highest level of selenium (treatment 10) dropped from 50 percent production in Period 1 to 25 percent production in Period 2. Carlson et al. (1954) reported that sodium arsenite or arsanilic acid was partially effective in counteracting the toxic effects of sodium selenite in a corn-soybean type ration. Consequently, at the end of the first week in Period 3, arsanilic acid (90 gms/ton) was added to diet 10. At the end of a three-week period the birds showed no signs of coming back into production and the experimental feed was discontinued.

Addition of fat or oil was without significant influence on bloodspot incidence (treatments 11 - 16). There was a consistent decrease in bloodspots with treatments 12 through 16, but it is doubtful if it can be attributed to the type of fat or oil in the diet since the

birds on the ration containing no added fat (treatment 16) also showed a slight decrease. Pope (1959) obtained a non-significant decrease in bloodspot incidence when crude cottonseed oil was included in the ration.

For treatments 1 through 9, a greater percentage of the bloodspots were of the larger size (over 4.8 mm) in Period 3 than in Period 1. This was not true for treatments 11 through 16 (Appendix Table 5). When the data were examined for each strain in Group 1 (Appendix Table 7), all strains were approximately equal in the tendency to produce a greater percentage of bloodspots over 4.8 mm in Period 3 as compared with Period 1.

The percentage production for Group 1 for Periods 1 and 3 for all birds laying in either Period is given in Table 5. Strain differences were noted but dietary treatments were without significant influence on production. It was noted, however, that the decline in production from Period 1 to Period 3 was not as great for the treatments containing vitamin B_{12}.

In comparing treatments in Group 2, strain differences in egg production (Appendix Table 8) were noted in Period 1; however, these differences did not continue throughout the entire experiment. An analysis of variance (Appendix Table 9) indicated that treatment effects were significant during Period 3. Egg production during Period 3 for the birds on the highest level of selenium (treatment 9) was significantly depressed. There was a non-significant increase in production for the birds on the control ration and the ration containing a low level of selenium with the latter resulting in the greatest increase.

Effects of treatment on production were not significant (Table 9)

for the birds in Group 3. Production was significantly lower in Period 5 than in Period 1 and 3.

Table 10 contains the hematocrit values for birds in Group 1. An analysis of variance was conducted on data of all periods individually. It was noted that there were strain and treatment differences during Period 1 (Appendix Table 11). Treatment differences were not to be expected since all birds were on the basal ration during this period, and treatments were actually replicates.

Treatment and strain differences were not significant at the $P < .01$ level during Period 3 but were significant at the $P < .05$ level. During Period 5 only strain differences were evident. The strain with the highest incidence of bloodspots (Strain A) had the lowest hematocrits. However, examination of the data did not indicate a correlation between bloodspot incidence and hematocrit values.

Since the hematocrit values during Period 3 decreased, but not significantly, as the vitamin B_{12} level was increased, it suggests that continued use of high levels of vitamin B_{12} may cause a decrease in erythrocyte formation. Hematocrit values for birds on the high level of cobalt (treatment 6) were significantly depressed during Period 3 when Periods 1 and 3 were compared for treatment 6 alone. The low level of cobalt (treatment 5) did not cause a significant increase in hematocrit values. Davis et al. (1945) reported that continued administration of a "high" level of cobalt to ducks depressed erythropoietic activity.

The fact that hematocrit values for all treatments except treatment 5 were lower during Period 5 is not readily explainable. Cohn and D'Amour (1951) reported that an increase in altitude stimulated

erythropoietic activity. For this reason, hourly station barometric pressure values were obtained from the weather bureau during each period on the days that blood was collected for hematocrit value determinations. Barometric pressure values were not different between periods.

Doupe et al. (1957) reported an increase in plasma volume in humans in summer months and hematocrit values fluctuated less than plasma volume but were generally lower in summer than in winter.

In order to determine the size of bloodspots and percent of bloodspots that are normally detected by a professional grader, a total of 360 eggs was taken to a commercial processing station. The grades obtained are included in Table 12.

After the eggs had been graded, they were broken out and evaluated. Twenty-five bloodspots (6.9%) were observed when broken out as opposed to 8 (2.2%) observed by the grader. Two of the eggs rejected by the grader because of bloodspots did not have bloodspots but had other abnormalities that could be mistaken for bloodspots. Of the remaining 6 eggs rejected by the grader, 5 of the eggs had bloodspots over 4.8 mm in size and 1 egg had a bloodspot between 1.6 and 4.8 mm.

Experiment B: Procedure and Results

The effects of BAPN (beta-aminopropionitrile), vitamin K, sulfaquinoxaline, reserpine, soybean-corn oil blend, reserpine plus sulfaquinoxaline, sulfaquinoxaline plus soybean-corn oil, vitamin K plus sulfaquinoxaline, and alfalfa leaf meal plus animal fat were studied with respect to bloodspot incidence in an experimental plan similar to that in Experiment A. Periods 1, 3 and 5 were 28 days in length while Periods 2 and 4 were 14 days in length. Eggs were collected five days each week.

There were 24 birds per treatment; 8 birds from each of the three strains previously described. Bloodspot incidence was measured for 28 days and birds were then allotted to the experimental treatments so as to balance initial bloodspot incidence.

Data from treatment 3 are not presented because the birds were not kept on the experimental diet the allotted time.

During Periods 4 and 5 the vitamin K level was increased from 4 to 40 mg/lb in treatment 5 and the sulfaquinoxaline from 0.0125 to 0.1 percent in treatments 6 and 11. A total of 17, 242 eggs were examined in this experiment and the data is summarized in Tables 15 through 23 and Appendix Tables 14 through 23.

The calendar dates for this experiment were as follows:

Period 1 - January 9 to February 5

Period 2 - February 6 to February 19

Period 3 - February 20 to March 18

Period 4 - March 19 to April 2

Period 5 - April 3 to April 29

Hematocrit values were determined in only one period of this

experiment because the birds began to decrease in production after being handled for the collection of blood. Since egg production is essential in order that bloodspot incidence can be determined, collection of blood for hematocrit determinations was discontinued.

Table 13. Composition of the Basal Ration Fed in Experiment B

Ingredient	Amount per Cwt.
Corn yellow No. 2, ground	70.00 lbs.
Soybean oil meal, 44% protein	19.00 "
Meat and bone scrap, 50% protein	3.00 "
Fish meal with solubles, 60% protein	1.25 "
Dicalcium phosphate, feed grade	2.00 "
Limestone, ground	5.00 "
Salt, iodized	.25 "
Manganese sulfate, 70% feed grade	.025 "
Riboflavin suppl. (16 gm/lb)	1 gram
Vitamin A (10,000 IU/gm)	20 "
Vitamin D (3,000 ICU/gm)	10 "
Total	100.00 lbs. +

Calculated analysis:

Crude protein	%	17.0	Vitamin B_{12}	(mcg.lb)	1.3
Crude fat	%	3.1	Riboflavin	(mg/lb)	1.1
Crude fiber	%	3.0	Niacin	(mg/lb)	10.0
Calcium	%	2.8	Pantothenic acid	(mg/lb)	2.8
Phosphorus	%	.8	Choline	(mg/lb)	407
Vitamin D	(ICU/lb)	300	Energy	(Cal/lb)	945
Vitamin A	(IU/lb)	3050			

Table 14. Changes in Basal Ration for Experiment B

Ingredient	Units	Diets[1]											
		1	2	3	4	5	6	7	8	9	10	11	12
BAPN	percent	--	.03	.09	--	--	--	--	--	--	--	--	--
Vitamin K	mg/lb	--	--	--	2.0	4.0	--	--	--	--	2.0	--	--
Sulfaquinoxaline	percent	--	--	--	--	--	.0125	--	.0125	--	--	.0125	--
Reserpine	mg/lb	--	--	--	--	--	--	2.0	2.0	--	--	--	--
Soybean-corn oil[2]	percent	--	--	--	--	--	--	--	--	5.0	5.0	5.0	--
Alfalfa leaf meal	percent	--	--	--	--	--	--	--	--	--	--	--	1.3
Animal fat	percent	--	--	--	--	--	--	--	--	--	--	--	1.5

[1] Changes in the percentages of corn were made to compensate for additions of oil, fat or alfalfa leaf meal.

[2] A blend of refined soybean and corn oil in a 4:1 ratio.

Table 15. Change in Bloodspot Incidence Between Periods 1 and 3 When Calculated on an Individual Bird Basis

Treatment	Average percent change	Percentage of birds increasing	Percentage of birds decreasing	Percentage of birds showing no change	Chi square values
1	+ 6	57	19	24	3.14
2	- 5	38	38	24	1.10
3	--	--	--	--	--
4	- 1	45	45	10	1.11
5	+ 1	50	45	5	.50
6	- 6	33	48	19	1.06
7	+ 5	45	40	15	.32
8	+ 8	59	35	6	1.73
9	- 4	48	33	19	.19
10	+ 1	48	30	22	3.45
11	0	48	38	14	.06
12	+ 2	43	38	19	.19
			Total		12.85

Chi-square value for 20 degrees of freedom ($P < .01$) = 37.57

Table 16. Change in Bloodspot Incidence Between Periods 3 and 5 Calculated on an Individual Bird Basis

Treatment	Average percent change	Percentage of birds increasing	Percentage of birds decreasing	Percentage of birds showing no change	Chi square values
1	- 5	26	53	21	1.57
2	+ 2	43	38	19	.12
3	--	--	--	--	--
4	+ 9	57	33	10	2.59
5[a]	- 3	40	55	5	3.33
6[b]	0	38	37	25	.62
7	+ 1	37	42	21	.17
8	- 7	29	53	18	1.03
9	+ 6	52	29	19	1.65
10	+ 7	50	32	18	1.03
11[b]	- 1	32	58	10	2.34
12	+ 8	35	35	30	2.17
			Total		16.62

Chi-square value for 20 degrees of freedom ($P < .01$) = 37.57

[a] Vitamin K increased to 40 mg/lb during Period 5

[b] Sulfaquinoxaline increased to 0.1% during Period 5

Table 17. The Effect of Handling of Birds for Collection of Blood for Hematocrit Determinations on the Incidence of Bloodspots

	Three days before			Three days after		
	No. of eggs	No. of bloodspots	%	No. of eggs	No. of bloodspots	%
Handled	61	13	21	64	12	19
Not handled	72	20	28	71	15	21

Table 18. The Effect of Handling Birds and Treating for Mites on the Incidence of Bloodspots

	Three days before			Three days after		
	No. of eggs	No. of bloodspots	%	No. of eggs	No. of bloodspots	%
Handled	254	57	22	216	36	17
Not handled	245	56	23	242	62	26
Handled	246	50	20	246	52	21
Not handled	217	40	18	213	36	17

Table 19. Analysis of Variance of Prothrombin Times Obtained During Period 5

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	3	9.3	.42	5.95
Strain	2	10.0	.44	6.93
T X S	6	14.8	.65	4.82
Error	12	22.5		

Average prothrombin times:

Treatment	Seconds
1	28.8
5	31.7
6	31.1
11	31.1

Strains:

A	30.0
B	31.9
C	30.0

Table 20. Analysis of Variance of Hematocrit Values

Source of variation	Degrees of freedom	Mean square	F	F .01
Strains	2	304.37	32.34	4.71
Within	174	9.41		

Strains:	A	B	C
	25.1	27.9	29.6

Means not underscored by the same line are significantly different ($P < .01$).

Table 21. Percentage Egg Production for Periods 1 and 3

Treatment	Period 1	Period 3	Amount of change
	%	%	
1	73	70	- 3
2	71	62	- 9
3	--	--	--
4	72	70	- 2
5	69	61	- 8
6	72	62	- 10
7	74	56	- 18
8	73	52	- 21
9	70	63	- 7
10	72	65	- 7
11	78	62	- 16
12	75	73	- 2

Table 22. Percentage Egg Production for Periods 3 and 5

Treatment	Period 3	Period 5	Amount of change
	%	%	
1	70	61	- 9
2	62	66	+ 4
3	--	--	--
4	70	59	- 11
5	61	60	- 1
6	62	50	- 12
7	56	65	+ 9
8	52	60	+ 8
9	63	64	+ 1
10	65	66	+ 1
11	62	45	- 17
12	73	67	- 6

Table 23. Analysis of Variance of Egg Production Data for Periods 1, 3 and 5

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	10	26.1	2.14	2.35
Periods	2	366.0	29.97	4.65
Strains	2	132.0	10.81	4.65
T X P	20	26.3	2.15	1.91
T X S	20	18.3	1.50	1.91
P X S	4	26.7	2.19	3.35
T X S X P	40	16.2	11.33	1.63
Error	607	12.2		

Since T X P interaction was significant, an analysis of variance was conducted on data of all periods individually in order to determine if treatments had a significant effect within each period. Analysis of variance tables for individual periods are in the Appendix.

Discussion of Experiment B

The various experimental diets were without significant effect on the percentage of eggs containing bloodspots (Appendix Table 14) or on the number of birds increasing or decreasing in the production of bloodspot eggs (Table 15). Pope et al. (1961) also failed to affect bloodspot incidence when vitamin K was included in the diet. Pope (1959), however, obtained a non-significant decrease in bloodspots when crude cottonseed oil was fed. In Experiment A, of the present study, the inclusion of crude cottonseed oil or a refined soybean-corn oil blend produced a non-significant decrease in incidence of bloodspots but the effect was not as great when repeated in Experiment B (treatment 9).

Between Periods 1 and 3, in general, Strain A showed a decrease regardless of treatment, Strain B showed an increase, and Strain C was inconsistent in bloodspot incidence (Appendix Table 32). Between Periods 3 and 5 no consistent tendency of strains to increase or decrease was apparent (Appendix Table 17).

In comparing Periods 1 and 3, or 3 and 5, the percentage of bloodspots falling in each size category (Appendix Table 18) was approximately the same for each period.

During Periods 1 and 3, a slightly higher percentage of the eggs from birds laying at a rate of 50 percent or more contained bloodspots than the eggs from birds laying at a rate less than 50 percent. The percent bloodspots for the less than 50 percent group declined slightly from Periods 1 to 5. This can be explained on the basis that in Period 5 a smaller percentage of the eggs were from the birds with high bloodspot incidence (Strain A).

From Tables 17 and 18, it can be seen that handling birds for

collection of blood or for treatment of mites had no effect on the incidence of bloodspots within a three-day period following handling. Frightening hens at different hours throughout the day (Jeffrey and Pino, 1943) or trapnesting (Stiles and Dawson, 1959) had no influence on the incidence of bloodspots.

During Period 5, prothrombin times, determined for six birds from each of four treatments (1, 5, 6 and 11), were not different due to strain or treatments (Table 19).

Hematocrit differences between strains were significantly different (Table 20).

Significant strain differences in egg production were noted during Period 1 (Appendix Table 20). During Period 1, treatments were actually replicates since all birds were on the basal ration. No differences between treatments existed.

There were differences in egg production due to strain and treatment during Period 3 (Appendix Table 21). Birds on treatment 8, which contained sulfaquinoxaline and reserpine, produced significantly less eggs than birds on the control ration. Birds on treatment 12, which contained dehydrated alfalfa meal and animal fat, produced significantly more eggs than those birds on treatments 8 or 7 (reserpine alone).

During Period 5, egg production for the birds on the soybean-corn oil blend plus sulfaquinoxaline (treatment 11) was lower than egg production for the birds on the control ration but not significantly so (Appendix Table 22). Egg production for treatments 7, 9, 10, 2 and 12 was significantly higher than treatment 11. This was not true for the birds on sulfaquinoxaline alone (treatment 6).

GENERAL DISCUSSION

As previously mentioned, the incidence of bloodspots in chicken eggs is influenced considerably by heredity and much progress has been made in overcoming this problem by the selection of proper breeding stock. Nevertheless, thus far, genetics has not completely solved this problem because a small percentage of the eggs being marketed today still contain this defect. This indicates that other factors may also be involved.

How should the effect of treatment on incidence of bloodspots be measured? Numerous workers in the past have used the technique of comparing the pre-experimental period with the experimental period but generally on a group or pen basis. By maintaining birds in individual cages it was possible to observe the effect of treatment on the individual bird and determine the number of birds that were influenced in a like manner. When group comparisons are made with respect to bloodspot incidence, the change in incidence between pre-experimental and experimental period may be caused by one or a few birds -- but the change is attributed to the entire group. Thus, it is possible for a few individuals to bias the data. In addition, when individual comparisons are made, this automatically takes into consideration only those birds that lay in both the pre-experimental and experimental period, thus eliminating the influence of any particular bird that lays in only one period.

Bearse (1958) reported an increase in bloodspot incidence when reserpine was included in the diet. He based his conclusions on a comparison of the birds on the reserpine-supplemented diet with that of the birds on the control ration with respect to change in incidence for both groups between the pre-experimental and experimental period. For

example, the birds on the reserpine-supplemented diet showed an increase of two percent while the birds on the control ration showed no change. (Presumably this was on a group basis).

If using this type of comparison as the sole criterion for evaluation is valid, then reserpine in this study had a beneficial effect because the birds fed the reserpine did not show as great an increase in bloodspot incidence as did those fed the control ration (when pre-experimental and experimental periods were compared). On the basis of the same type comparison, BAPN and sulfaquinoxaline were even more effective in reducing the incidence of bloodspots. Admittedly, experimental rations must be compared with the control rations but not without also comparing the pre-experimental period with the experimental period for any particular ration and determining if the difference between periods is significant. It would appear that the most valid test for determining if the difference between periods is significant is to determine the number of birds influenced in a similar manner.

Another type of comparison that could be made would be to adjust the values for the control ration to zero and establish comparsions of the experimental rations with the control ration on this basis. For example, birds in Experiment A on the control ration showed an average increase in bloodspot incidence of 6 percent while the birds on treatment 2 showed an average decrease of 3 percent, or a difference of 9 percent when treatment 2 is compared with the control ration. This type of comparison, however, has the disadvantage in that it does not take into consideration the fact that if the pre-experimental period is not compared with the experimental period for any particular ration than those birds do not act as their own controls.

Examination of the data for individual birds in this study indicated that the birds could be divided into three types on the basis of bloodspot incidence. The first type laid eggs that contained no bloodspots; the second type produced eggs infrequently that contained bloodspots and the third type produced eggs that contained a high percentage of bloodspots. By determining bloodspot incidence at stated intervals for a short period of time, it is conceivable, that at the first "break-out", a portion of the eggs examined would be from hens of the third type while at the second "break-out", not any of the eggs would be from birds of this type. Consequently, it would be erroneous to draw the conclusion that treatment caused a decrease in bloodspot incidence on the basis of such experimental procedure. An example of determining bloodspot incidence at stated intervals is the work of Berruti and Dedrick (1960). Bloodspot incidence was determined at 2- and 4-week intervals after initiation of experimental diets.

Since vitamin K is necessary for prothrombin formation which in turn is directly involved in blood clotting, much attention has been given to vitamin K as being a factor in reducing the incidence of bloodspots. Blood clotting does not normally occur until the blood has been released from the blood vessel. Consequently, it would appear that any influence of vitamin K would be on size, rather than on number, of bloodspots. Nalbandov and Card (1944), Denton (1947) and Pope et al. (1961) found no effect on number of bloodspots produced when vitamin K was included in the diet.

Why, then, study the influence of vitamin K on bloodspot incidence when numerous reports have indicated no effect? Day (1960) reported that vitamin K (menadione sodium bisulfite) was not stable when present

in a calcite carrier and Pope and others used vitamin K in such a carrier. In addition, Berruti and Dedrick reported that by feeding a more stable vitamin K they were able to cause a considerable reduction in the incidence of bloodspots.

Since sulfaquinoxaline had been suggested by Finkel (1961) to be an antagonist of vitamin K, it was included in this study to accentuate the vitamin K-deficient effect, if any.

The findings of this study, which do not agree with those of Berruti and Dedrick, are supported by the fact that sulfaquinoxaline had no influence on bloodspot incidence when included in a vitamin K-low diet. Thus, it is evident that vitamin K per se is not the solution to the bloodspot problem.

The fact that sulfaquinoxaline did not have any influence on prothrombin times is probably due to the low level used. Cuckler and Ott (1955) reported that at least 0.4 percent was required to produce an increase in prothrombin time. The use of higher levels by this author resulted in cessation of egg production. Consequently, a correlation between prothrombin time and bloodspot incidence could not be determined.

Does hemorrhaging other than that caused by a vitamin K deficiency occur, and, if so, what would be the effect of this type of hemorrhaging on the incidence of bloodspots? Waibel and Pomeroy (1958) reported that BAPN produced "dissecting aneurysm of the posterior aorta and consequent death due to internal hemorrhage" in growing turkeys. Barnett et al. (1958) reported that supplemental vitamin K did not consistently reduce BAPN-induced hemorrhaging in chickens, indicating that the BAPN effect is not mediated by causing a vitamin K deficiency.

Since BAPN did not affect the number or size of bloodspots produced

it is possible that the effect of BAPN on the small veins and capillaries of theovary differs from the effect on larger vessels.

High levels of vitamin B_{12} had no influence on the incidence of bloodspots. Pope (1959) obtained a significant increase in bloodspot incidence by feeding high levels of vitamin B_{12} (30 mcgs/lb). However, he also noted that the feeding of vitamin A at levels of 3,575 or 10,215 USP units/lb counteracted the detrimental effects of this high level of vitamin B_{12}. The vitamin A level of the basal ration used in the work discussed here was 3,563 units/lb which may explain why the use of higher levels of vitamin B_{12} than Pope fed did not increase bloodspot incidence.

Hematocrit values for the birds on the high levels of vitamin B_{12} were normal which indicated that a condition of erythrocytosis did not exist. Yet, the literature states that erythrocytosis can be induced by the administration of "excessive" doses of vitamin B_{12} or other cobalt compounds with a resultant increase in peripheral resistance which may in turn lead to hypertension (Kugelmass, 1959). Weiss (1958) reported that incidence of bloodspotting was higher in a high blood pressure line of birds than in a low blood pressure line, but not significantly so.

If hypertension is associated with bloodspot incidence as suggested by Weiss, would the feeding of vegetable oils and animal fats result in different effects on the number of bloodspots produced? Daghir et al. (1960) reported that soybean oil caused a significant decrease in serum cholesterol levels while white grease was without effect. Since the characteristic lesion of the atherosclerotic artery may contain up to 70 percent cholesterol, it is believed that hypercholesterolemia and

atherosclerosis are associated in some way (Best and Taylor, 1961).

In humans, a narrowing of the peripheral vessels, such as would happen when a sclerotic lesion occurs in an artery, can result in hypertension. Although narrowing of the larger peripheral vessels may result in hypertension, the pressure in the small veins and capillaries does not change materially. Nalbandov and Card (1944) suggested that bloodspots may be due to rupture of small blood vessels or capillaries. However, it does not appear that the rupture would be the result of increased blood pressure resulting from a condition such as atherosclerosis. Thus, influencing blood cholesterol would not be expected to influence the number of bloodspots produced. In addition, the feeding of reserpine, an anti-hypertensive agent, had no influence on number or size of bloodspots produced.

There is a positive correlation between whole blood specific gravity and hematocrit values (Kugelmass, 1959), therefore, any disturbance that would affect the "corpuscle:plasma volume ratio" would influence hematocrit values. For example, after hemorrhage there is a fall in the corpuscle:plasma volume ratio due to an increase in the return of fluid from the tissue spaces. The blood loss from hens with high bloodspot incidence was possibly greater than the loss from hens with low bloodspot incidence but it is doubtful if this loss was enough to affect the corpuscle:plasma volume ratio, and consequently, would not explain why hematocrit values were lowest for the birds with the highest incidence of bloodspots.

Acute disease increases whole blood specific gravity, however, mortality was highest for the strain of birds with the lowest hematocrit values.

That the high level of cobalt appeared to depress erythrocyte formation as evidenced by significantly lower hematocrit values, is in agreement with Davis et al. (1945) who stated that continued administration of "excessive" amounts of cobalt decreased the erythrocyte count in ducks.

The influence of vitamin B_{12} on egg production suggests re-evaluation of the vitamin B_{12} requirement of the laying hen for optimum egg production. The levels used were considerably higher than the present recommendations of the National Research Council.

As evidenced by a depression in egg production, the higher levels of selenium were toxic. On the other hand, the addition of a low level of selenium appeared to cause an increase in production. This suggests further study on the selenium requirement of laying hens. Poley et al. (1940) reported that better growth was obtained in chicks when 2 or 5 ppm of selenium was supplied in selenium-containing grains.

CONCLUSIONS

Two experiments were conducted using 600 Single Comb White Leghorn pullets from three different strains to determine the effect of certain dietary ingredients on the incidence of bloodspots in chicken eggs. Bloodspot incidence or size of bloodspots of individual birds before, during and after feeding the experimental rations was the criteria used to evaluate the dietary treatments employed.

The following dietary additives did not affect the number or size of bloodspots.

Vitamin B_{12} (15, 30 and 60 mcg/lb)

Cobalt, as $CoCO_3$ (1.32 and 90 mcg/lb)

Selenium as $Na_2SO_3 \cdot 5H_2O$ (1 and 5 ppm)

Crude cottonseed oil (5.0%)

Crude corn oil (5.0%)

Refined soybean-corn oil in a 4:1 ratio (5.0%)

Fat, animal, No. 2 yellow grease (none or 5.0%)

Beta-aminopropionitrile fumarate (BAPN) (0.03%)

Vitamin K as menadione sodium bisulfite (2,4 and 40 mg/lb)

Sulfaquinoxaline (0.0125% and 0.1%)

Reserpine (2 mg/lb)

Reserpine plus sulfaquinoxaline (2 mg/lb plus 0.0125%)

Refined scybean-corn oil plus vitamin K (5.0% plus 2 mg/lb)

Refined soybean-corn oil plus sulfaquinoxaline (5.0% plus 0.0125 or 0.1%)

Alfalfa leaf meal plus animal fat (1.3% plus 1.5%)

Subtraction from the diet of all the above had no effect on incidence of bloodspots.

Thus the hypothesis that the presence or absence of certain

dietary ingredients might influence the incidence of bloodspots in chicken eggs was not confirmed.

Egg production was significantly depressed by:

Selenium (5 ppm)

Reserpine (2mg/lb) plus sulfaquinoxaline (0.0125%)

Sulfaquinoxaline (0.1%) plus refined soybean-corn oil blend (5.0%)

Egg production was depressed, but not significantly, by:

Beta-aminopropionitrile fumarate (0.03%)

Reserpine (2 mg/lb)

Sulfaquinoxaline (0.1%)

Egg production was increased, but not significantly, by:

Vitamin B_{12} (60 mcg/lb)

Selenium (1 ppm)

Hematocrit values were significantly different between strains of birds with Strain A being lower than Strains B and C in Experiment A. In Experiment B, all strains were significantly different from each other (ranking low to high: Strain A, Strain B and Strain C).

In Experiment A, hematocrit values for July were significantly lower than for March and May. The hematocrit values for all treatments were lower during July, except for the treatment that had contained the low level of cobalt during May.

LITERATURE CITED

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

Balloun, S. L. and E. L. Johnson, 1952. Underheated soybean oil meal increases blood clotting time of chicks. Poultry Sci. 31:905 (Abs.)

Barnett, B. D., 1960. The effect of reserpine on artificially produced and spontaneously appearing aortic rupture. The Second Conference on the use of reserpine in poultry production. pp. 9-14.

Barnett, B. D. and C. L. Morgan, 1959. The effect of high levels of dietary fat on beta-aminopropionitrile induced internal hemorrhage in chicks. Poultry Sci. 38:589-593.

Barnett, B. D., D. J. Richey and C. L. Morgan, 1958. The effect of anti-coagulants on toxicity of beta-aminopropionitrile. Poultry Sci.37: 1124-1128.

Bearse, G. E., 1958. How nutrition and management affect bloodspots in eggs. 11th Washington State College Animal Industries Conference Abstracts.

Bearse, G. E. and L. R. Berg, 1958. The fat soluble vitamins and bloodspot incidence. Poultry Sci. 37:1184 (Abs.)

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

Bearse, G. E., C. F. McClary and H. C. Saxena, 1960. Bloodspot incidence in chicken eggs and vitamin A level of the diet. Poultry Sci. 39: 860-865.

Berruti, R. and G. T. Dedrick, 1960. Evidence on the value of stabilized Heterogen K in reducing the incidence of bloodspots. Hetero-

chemical Corp. Unpub.

Best, C. H. and N. B. Taylor, 1961. The Physiological Basis of Medical Practice, 7th ed. The Williams and Wilkins Co., Baltimore, Md.

Card, L. E. and A. Nalbandov, 1944. Controlling blood and meat spots. Poultry Sci. 23:551 (Abs.)

Carlson, C. W., E. Guenthner, W. Kohlmeyer and O. E. Olson, 1954. Some effects of selenium, arsenicals and vitamin B_{12} on chick growth. Poultry Sci. 33:768-774.

Carver, J. S. and W. Henderson, 1941. The effect of rutin and ascorbic acid and of alfalfa on blood and meat spots in hens' eggs. Poultry Sci. 27:656 (Abs.)

Cohn, E. W. and F. E. D'Amour, 1951. Effects of high altitudes on growth and polycythemia in rats. Am. J. of Physiol. 161:394-399.

Cuckler, A. C. and W. H. Ott, 1955. Tolerance studies on sulfaquinoxaline in poultry. Poultry Sci. 34:867-879.

Daghir, N. J., W. W. Marion and S. L. Balloun, 1960. Influence of dietary fat and choline on serum and egg yolk cholesterol in the laying chicken. Poultry Sci. 39:1459-1466.

Davis, J. A., A. W. McCullough and R. H. Rigdon, 1945. Polycythemia produced by cobalt in the duck. J. of Lab. and Clin. Med. 30:327-336.

Day, E. J. and B. Glick, 1960. Chemical and biological assays of vitamin K premixes. Presented at the Informal Poultry Nutrition Conference in Chicago.

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

Doupe, J., M. H. Ferguson, and J. A. Hildes, 1957. Seasonal fluctuations

in blood volume. Can. J. Biochem. and Physiol. 35:203-213.

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

Farnsworth, G. M. and A. W. Nordskog, 1955. Estimates of genetic parameters influencing blood spots and other economic traits of the fowl. Poultry Sci. 34:1192 (Abs.)

Finkel, M. J., 1961. Vitamin K_1 and the vitamin K analogues. Clin. Pharmacol. and Therap. 2:794-814.

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

Hare, J. H., G. C. Anderson, C. E. Weakley, Jr. and J. K. Bletner, 1953. Factors contributing to a hemorrhagic condition in experimental chicks fed simplified rations. Poultry Sci. 32:904 (Abs.)

Harms, R. H. and F. R. Tarver, Jr., 1957. The influence of dicumarol upon blood clotting time and blood loss of young chickens. Poultry Sci. 36:76-79.

Houssay, B. A., 1955. Human Physiology, 2nd ed. McGraw-Hill Book Co., Inc., New York.

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

Jones, A. R., 1956. A device for rapidly deriving the hematocrit of blood centrifuged in ungraduated tubes. New England Jour. of Med. 254:172-174.

Kugelmass, N. I., 1959. Biochemistry of Blood in Health and Disease. Chas. C. Thomas Co., Springfield, Ill.

Lerner, M. I., L. W. Taylor and D. C. Lowry, 1951. Selection for increased incidence of bloodspots in White Leghorns. Poultry Sci. 30:748-757.

McGovern, J. J., A. R. Jones and A. G. Steinberg, 1955. The hematocrit of capillary blood. New England Jour. Of Med. 253:308-312.

Munro, S. S., 1952. Effect of different feeding formulas on blood-spotting. Poultry Sci. 31:929 (Abs.)

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

Nalbandov, A. V. and L. E. Card, 1947. The problem of blood and meat spots in chicken eggs. II. Its importance in poultry flocks, and a study of the nutritional factors involved. Poultry Sci. 26:400-409.

Perdue, H. S., J. A. Kolar and D. V. Frost, 1961. Non-correlation of vitamin K status and production of eggs with bloodspots. Poultry Sci. 40:1443 (Abs.)

Poley, W. E., W.O. Wilson and A. L. Moxon, 1940. The effect of selenized grains on the rate of growth of chicks. Poultry Sci. 19:358 (Abs.)

Pope, C. W., 1959. The effect of certain nutrients and other materials upon the incidence of bloodspots in chicken eggs. Ph. D. Thesis. Michigan State University.

Pope, C. W., P. J. Schaible and L. E. Dawson, 1961. Effects of certain nutrients upon bloodspots in chicken eggs. Poultry Sci. 40:377-382.

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

Roy, D. B. and H. R. Bird, 1959. Stimulation of chick growth by proline.

Poultry Sci. 38:192-196.

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

Snedecor, G. W., 1956. Statistical Methods. 5th ed. Iowa State College Press, Ames, Iowa.

Speckmann, E. W. and R. K. Ringer, 1961. Hemodynamic responses following reserpine feeding to turkeys. Poultry Sci. 40:1292-1298.

Stephens, J. F. and R. L. Tugwell, 1960. Sources and levels of vitamin K in relation to cecal coccidiosis. Poultry Sci. 39:1183-1187.

Stiles, P. G. and L. E. Dawson, 1959. The effect of physical disturbance, sound and light on the incidence of blood and meat spots and other egg quality factors. Poultry Sci. 38:1250 (Abs.)

Stiles, P. G., R. K. Ringer and L. F. Wolterink, 1958. A procedure for labeling bloodspots in chicken eggs with radioactive phosphorus. Poultry Sci. 37:600-601.

Sturkie, P. D., 1954. Avian Physiology. Comstock Publishing Associates, Ithaca, New York.

Sturkie, P. D., 1959. Cardiovascular effects of reserpine on the fowl. Conference on the use of the tranquilizing and antihypertensive agent Serpasil in animal and poultry production. pp. 18-20.

Titus, H. W., 1955. The Scientific Feeding of Chickens. 3rd. ed. The Interstate, Danville, Ill.

Travis, H. F., 1960. Nutritional studies on ranch-raised mink. Ph. D. Thesis, Michigan State University.

Waibel, P. E. and B. S. Pomeroy, 1958. Studies on the production of aortic hemorrhage in growing turkeys with beta-aminopropionitrile.

Poultry Sci. 37:934-938.

Weiss, H. S., 1958. Blood pressure and egg formation. Poultry Sci. 37: 33-36.

Yacowitz, H., R.D. Carter and E. Ross, 1955. Further studies on hemorrhagic syndrome induced by feeding high levels of sulfaquinoxaline to chicks. Poultry Sci. 34:1229 (Abs.)

Appendix Table 1. Bloodspot Incidence Expressed as the Percentage of Eggs Laid During Periods 1 and 3

Treatment	Period 1	Period 3	Amount of change	"t" values
	%	%	%	
1	21	16	- 5	.42
2	20	19	- 1	.08
3	24	17	- 7	.52
4	23	18	- 5	.39
5	32	23	- 9	.67
6	19	21	+ 2	.16
7	7	10	+ 3	.30
8	6	4	- 2	.22
9	8	9	+ 1	.09
11	8	10	+ 2	.18
12	11	7	- 4	.11
13	8	5	- 3	.34
14	12	9	- 3	.27
15	11	8	- 3	.30
16	6	4	- 2	.23

Appendix Table 2. Bloodspot Incidence Expressed as the Percentage of Eggs Laid During Periods 3 and 5

Treatment	Period 3	Period 5	Amount of change	"t" values
	%	%	%	
1	16	17	+ 1	.08
2	17	21	+ 4	.29
3	17	16	- 1	.08
4	14	21	+ 7	.52
5	21	24	+ 3	.21
6	20	25	+ 5	.36
7	10	6	- 4	.37
8	4	3	- 1	.13
9	9	7	- 2	.16
11	5	4	- 1	.12
12	8	6	- 2	.19
13	5	3	- 2	.28
14	9	8	- 1	.10
15	8	9	+ 1	.10
16	4	6	- 2	.23

Appendix Table 3. Percentage of Bloodspots by Strains Within Treatments for Periods 1 and 3 for Birds in Group 1

Treatment	Strain	Period 1	Period 3	Amount of change
		%	%	%
1	A	40	32	- 8
	B	12	10	- 2
	C	12	4	- 8
2	A	39	39	0
	B	18	20	+ 2
	C	10	6	- 4
3	A	61	51	-10
	B	10	5	- 5
	C	8	5	- 3
4	A	48	44	- 4
	B	17	15	- 2
	C	8	5	- 3
5	A	66	53	-13
	B	23	20	- 3
	C	8	6	- 2
6	A	52	50	- 2
	B	15	20	+ 5
	C	7	1	- 6

Appendix Table 4. Percentage of Bloodspots by Strains Within Treatments for Periods 3 and 5 for Birds in Group 1

Treatment	Strain	Period 3	Period 5	Amount of change
		%	%	%
1	A	33	34	+ 2
	B	10	21	+11
	C	4	6	+ 2
2	A	37	52	+15
	B	21	20	- 1
	C	6	10	+ 4
3	A	54	52	- 2
	B	5	7	+ 2
	C	5	11	+ 6
4	A	43	71	+28
	B	15	22	+ 7
	C	5	9	+ 4
5	A	52	66	+14
	B	22	15	- 7
	C	6	13	+ 7
6	A	50	54	+ 4
	B	19	23	+ 4
	C	1	4	+ 3

Appendix Table 5. Percentage of Bloodspots Falling Into Each Size Category During Periods 1 and 3

Treatment	Period 1 Size			Period 3 Size		
	Under 1.6 mm	Between 1.6-4.8 mm	Over 4.8 mm	Under 1.6 mm	Between 1.6-4.8 mm	Over 4.8 mm
	%	%	%	%	%	%
1	49	22	29	31	26	43
2	56	17	27	31	21	48
3	43	19	38	27	18	55
4	45	22	33	38	26	36
5	37	21	42	37	16	47
6	35	29	36	29	22	49
7	56	21	23	50	25	25
8	78	15	7	63	4	33
9	66	17	17	46	18	36
11	68	16	16	58	14	28
12	64	14	22	64	14	22
13	61	17	22	56	25	19
14	58	17	25	54	17	29
15	65	21	14	60	21	19
16	65	18	17	70	9	21

Appendix Table 6. Percentage of Bloodspots Falling Into Each Size Category During Periods 3 and 5

Treatment	Period 3 Size			Period 5 Size		
	Under 1.6 mm	Between 1.6-4.8 mm	Over 4.8 mm	Under 1.6 mm	Between 1.6-4.8 mm	Over 4.8 mm
	%	%	%	%	%	%
1	31	26	43	40	23	37
2	31	22	48	37	19	44
3	27	18	55	33	26	41
4	38	26	36	36	20	44
5	37	16	47	39	20	41
6	29	23	49	36	18	46
7	50	25	25	47	22	31
8	63	4	33	38	48	14
9	46	18	36	41	32	27
11	58	14	28	50	14	36
12	64	14	22	74	22	4
13	56	25	19	41	14	45
14	54	17	29	39	18	43
15	60	21	19	55	19	26
16	70	9	21	50	22	28

Appendix Table 7. Percentage of Bloodspots Falling Into Each Size Category by Strains Within Treatments During Periods 1 and 3 for Birds in Group 1

Treatment	Strain	Period 1 No. of spots	Period 1 Under 1.6 mm	Period 1 Between 1.6-4.8 mm	Period 1 Over 4.8 mm	Period 3 No. of spots	Period 3 Under 1.6 mm	Period 3 Between 1.6-4.8 mm	Period 3 Over 4.8 mm
			%	%	%		%	%	%
1	A	105	50	18	32	76	30	20	50
	B	28	46	29	25	24	29	46	25
	C	39	49	26	25	9	45	22	33
2	A	69	54	20	26	53	26	23	51
	B	48	44	19	37	58	32	25	45
	C	28	82	7	11	15	47	13	40
3	A	117	37	18	45	89	21	18	61
	B	20	80	0	20	11	55	9	36
	C	23	43	39	17	13	46	23	31
4	A	112	34	21	45	80	30	29	41
	B	47	53	30	17	52	38	27	35
	C	22	82	14	4	15	80	7	13
5	A	205	28	19	53	106	29	14	57
	B	62	57	32	11	51	41	37	22
	C	28	57	14	29	22	64	14	23
6	A	69	26	30	44	80	24	19	57
	B	48	40	27	33	53	36	30	34
	C	19	53	31	16	3	33	0	67

Appendix Table 8. Analysis of Variance of Egg Production Data for Birds in Group 2 for Period 1

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	2	68.0	.39	5.20
Strains	1	1633.0	9.24	7.33
T X S	2	52.0	.29	5.20
Error	39	176.0		

Strains	B	C
Av. eggs/ hen	30.1	42.2

Appendix Table 9. Analysis of Variance of Egg Production Data for Birds in Group 2 for Period 3

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	2	2368.0	11.91	5.29
Strains	2	516.0	2.58	7.44
T X S	2	105.5	.53	5.29
Error	34	199.4		

Treatments	9	7	8
Av. eggs/hen	24.5	44.2	50.7

Means not underscored by the same line are significantly different ($P < .01$).

Appendix Table 10. Analysis of Variance of Egg Production Data for Birds in Group 2 for Period 5

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	2	291.5	1.34	5.39
Strains	1	54.0	.25	7.56
T X S	2	655.0	3.02	5.39
Error	30	216.9		

Appendix Table 11. Analysis of Variance for Hematocrit Values for Birds in Group 1 for Period 1

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	5	21.44	3.42	3.20
Strains	2	44.06	7.05	4.82
T X S	10	3.89	.62	2.51
Error	109	6.25		

Treatments	5	2	1	3	4	6
Hct. values	27.1	28.1	28.4	28.4	29.4	29.8

Strains	A	B	C
Hct. values	27.2	29.0	29.1

Means not underscored by the same line are significantly different ($P < .01$)

Appendix Table 12. Analysis of Variance for Hematocrit Values for Birds in Group 1 for Period 3

Source of variation	Degrees of freedom	Mean square	F	F .05
Treatments	5	13.91	2.59	2.30
Strains	2	25.79	4.79	3.09
T X S	10	5.53	1.02	1.92
Error	105	5.38		

Treatments	6	5	1	4	3	2
Hct. values	27.3	28.1	28.5	28.6	29.4	29.7

Strains	A	B	C
Hct. values	27.6	28.8	29.1

Means not underscored by the same line are significantly different ($P < .05$).

Appendix Table 13. Analysis of Variance for Hematocrit Values for Birds in Group 1 for Period 5

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	5	4.95	1.02	3.25
Strains	2	39.11	8.11	4.88
T X S	10	6.79	1.41	2.55
Error	84	4.82		

Strains	A	B	C
Hct. values	25.4	27.4	27.5

Means not underscored by the same line are significantly different ($P < .01$).

Appendix Table 14. Bloodspot Incidence Expressed as the Percentage of Eggs Laid During Periods 1 and 3

Treatment	Period 1	Period 3	Amount of change	"t" values
	%	%	%	
1	13	19	+ 6	.54
2	29	24	- 5	.37
3	--	--	--	--
4	26	25	- 1	.08
5	22	20	- 2	.16
6	24	18	- 6	.48
7	19	20	+ 1	.08
8	20	25	+ 5	.35
9	14	13	- 1	.10
10	17	19	+ 2	.17
11	14	15	+ 1	.09
12	21	22	+ 1	.08

Appendix Table 15. Bloodspot Incidence Expressed as the Percentage of Eggs Laid During Periods 3 and 5

Treatment	Period 3	Period 5	Amount of change	"t" values
	%	%	%	
1	17	13	- 4	.35
2	24	26	+ 2	.15
3	--	--	--	--
4	24	28	+ 4	.30
5[a]	21	19	- 2	.17
6[b]	19	21	+ 2	.14
7	17	23	+ 4	.31
8	25	19	- 6	.43
9	14	17	+ 3	.29
10	19	24	+ 5	.40
11[b]	15	9	- 6	.57
12	22	26	+ 4	.30

[a] Vitamin K increased to 40 mg/lb during Period 5

[b] Sulfaquinoxaline increased to 0.1% during Period 5

Appendix Table 16. Percentage of Bloodspots by Strains Within Treatments for Periods 1 and 3

Treatment	Strain	Period 1	Period 3	Amount of change
		%	%	%
1	A	27	26	- 1
	B	7	15	+ 8
	C	8	16	+ 8
2	A	54	39	- 13
	B	31	34	+ 3
	C	9	5	- 4
4	A	55	43	- 12
	B	19	20	+ 1
	C	14	17	+ 3
5	A	36	35	- 1
	B	13	19	+ 6
	C	16	13	- 3
6	A	48	36	- 12
	B	18	17	- 1
	C	9	6	- 3
7	A	36	33	- 3
	B	7	18	+ 11
	C	13	13	0
8	A	51	39	- 12
	B	11	32	+ 21
	C	6	12	+ 6
9	A	31	30	- 1
	B	4	11	+ 7
	C	9	2	- 7
10	A	36	37	+ 1
	B	12	9	- 3
	C	5	12	+ 7
11	A	33	21	- 12
	B	7	13	+ 6
	C	6	12	+ 6
12	A	39	29	- 10
	B	16	25	+ 9
	C	12	12	0

Appendix Table 17. Percentage of Bloodspots by Strains Within Treatments for Periods 3 and 5

Treatment	Strain	Period 3	Period 5	Amount of change
		%	%	%
1	A	22	15	- 7
	B	15	16	+ 1
	C	16	8	- 8
2	A	39	51	+ 12
	B	34	31	- 3
	C	5	5	0
4	A	43	63	+ 20
	B	20	23	+ 3
	C	16	17	+ 1
5[a]	A	37	29	- 8
	B	19	18	- 1
	C	13	12	- 1
6[b]	A	35	40	+ 5
	B	17	13	- 4
	C	5	16	+ 11
7	A	24	37	+ 13
	B	18	19	+ 1
	C	13	18	+ 5
8	A	39	49	+ 10
	B	32	22	- 10
	C	12	1	- 11
9	A	33	38	+ 5
	B	14	15	+ 1
	C	2	9	+ 7
10	A	37	47	+ 10
	B	9	17	+ 8
	C	12	6	- 6
11[b]	A	21	7	- 14
	B	13	10	- 3
	C	12	11	- 1
12	A	33	35	+ 2
	B	25	35	+ 10
	C	12	8	- 4

[a] Vitamin K increased to 40 mg/lb during Period 5

b Sulfaquinoxaline increased to 0.1% during Period 5

Appendix Table 18. Percentage of Bloodspots Falling Into Each Size Category During Periods 1 and 3

Treatment	Period 1 Size			Period 3 Size		
	Under 1.6 mm	Between 1.6-4.8 mm	Over 4.8 mm	Under 1.6 mm	Between 1.6-4.8 mm	Over 4.8 mm
	%	%	%	%	%	%
1	56	24	20	56	18	25
2	46	24	30	41	13	46
3	--	--	--	--	--	--
4	46	18	36	44	14	42
5	52	17	32	50	16	34
6	60	23	17	43	17	40
7	47	22	31	61	12	27
8	52	17	31	53	18	29
9	58	5	37	55	21	24
10	59	16	26	44	20	36
11	49	19	32	49	18	33
12	57	14	29	56	20	24

Appendix Table 19. Percentage of Bloodspots Falling Into Each Size Category for Periods 3 and 5

Treatment	Period 3 Size			Period 5 Size		
	Under 1.6 mm	Between 1.6-4.8 mm	Over 4.8 mm	Under 1.6 mm	Between 1.6-4.8 mm	Over 4.8 mm
	%	%	%	%	%	%
1	62	15	23	60	23	17
2	40	13	48	49	18	33
3	--	--	--	--	--	--
4	42	15	43	48	17	36
5[a]	49	15	36	49	20	31
6[b]	41	16	43	56	3	41
7	70	11	19	52	14	34
8	53	18	29	46	13	41
9	56	19	25	44	20	36
10	40	20	36	48	16	36
11[b]	50	17	33	44	37	19
12	55	20	25	44	15	41

[a] Vitamin K increased to 40 mg/lb during Period 5

[b] Sulfaquinoxaline increased to 0.1% during Period 5

Appendix Table 20. Analysis of Variance of Egg Production Data for Period 1

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	10	4.9	.47	2.40
Strains	2	65.5	6.35	4.70
T X S	20	9.4	.92	1.96
Error	222	10.3		

Strains	C	A	B
Av. eggs/hen	13.8	14.3	15.5

Means not underscored by the same line are significantly different ($P < .01$)

Appendix Table 21. Analysis of Variance of Egg Production Data for Period 3

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	10	34.5	2.67	2.41
Strains	2	66.0	5.12	4.71
T X S	20	25.1	1.95	1.97
Error	198	12.9		

Treatments and Av. eggs/hen

8	7	5	11	2	6	9	10	1	4	12
10.5	11.2	12.2	12.3	12.4	12.5	12.6	13.0	13.9	13.9	15.3

Strains	A	C	B
Av. eggs/hen	11.6	12.7	13.4

Means not underscored by the same line are significantly different ($P < .01$)

Appendix Table 22. Analysis of Variance of Egg Production Data for Period 5

Source of variation	Degrees of freedom	Mean square	F	F .01
Treatments	10	39.3	2.60	2.28
Strains	2	54.0	3.58	4.71
T X S	20	16.2	1.07	1.97
Error	196	15.1		

Treatments and Av. eggs/hen

11	6	4	8	5	1	9	7	10	2	12
9.0	10.0	11.9	12.0	12.1	12.2	12.8	13.1	13.2	13.3	13.5

Means not underscored by the same line are significantly different ($P < .01$)

Appendix Table 23. Bloodspot Incidence for Birds at Two Levels of Production Without Regard for Treatment

Period	Strain	50% Production or greater			Less than 50% production		
		No. of eggs	No. of spots	Percent spots	No. of eggs	No. of spots	Percent spots
1		3577	756	21	124	30	24
3		2694	532	20	241	55	23
5		2542	542	21	243	53	22
1	A	1134	466	41	50	21	42
	B	1301	181	14	14	3	21
	C	1142	109	10	60	6	10
3	A	677	226	33	90	29	32
	B	1021	196	19	60	15	25
	C	996	110	11	91	11	12
5	A	652	256	39	77	29	38
	B	1018	203	20	43	11	26
	C	872	83	10	123	13	11