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Normal Levels for Hemoglobin, Phosphorus, Calcium, Carotene, and Vitamin A for Broad-Breasted Bronze Turkeys

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NORMAL LEVELS FOR HEMOGLOBIN, PHOSPHORUS, CALCIUM, CAROTENE, AND VITAMIN A FOR BROAD-BREASTED BRONZE TURKEYS.

by

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The introduction of micro methods has brought blood analyses into the limelight since the blood picture of normal subjects is rapidly becoming of primary importance in diagnosis of disease as well as a criteria of dietary deficiencies.

A review of the literature revealed a lack of data for blood levels of turkeys. Scott, Serfontein, and Seiling (1933) report analysis of blood samples from fifteen young Bronze turkeys. Nielsen and Madsen (1940) report serum calcium and phosphorus values for young normal turkeys. Dukes(1942) has included turkeys in his table of blood composition, but many of his values seem to be those reported earlier by Scott, Serfontein, and Seiling (1933). Rhian, Wilson, and Moxon (1944) have published blood levels for Broad-Breasted Bronze turkeys in their study on the relationship of fertility and hatchability to the concentration of certain blood constituents.

There is evidence that the composition of turkey blood is dependent on many factors--such as, nutrition, housing facilities, sex, breed, and other things (Ewing, 1943). This experiment was designed to establish normal levels for hemoglobin, calcium, inorganic phosphorus, carotene, and vitamin A of Broad-Breasted Bronze turkey breeding stock, having adequate housing facilities and rations, and to evaluate the relationship of these blood levels with respect to egg production, fertility, and hatchability.

EXPERIMENTAL

The turkey hens were divided into four different groups of 25 similar hens. The bases for the selection of the hens for similarity were egg production, fertility, and hatchability records of the parent stock. They were housed in a rammed earth building equipped with strawlofts and they had access to cobblestone yards on days when the temperature was above 15° F. The pens were 16 feet by 12 feet and there were 25 hens and 4 toms in each pen. The toms were rotated weekly between pens. The toms were lighted starting December 22 and the females were lighted starting December 29. Trapnests were used in order to determine the individual egg records for the hens.

Turkey hen as used in this paper when referring to this experiment is any female turkey. All of the turkey hens were eight to ten months old at the start of the experiment. The period for the study includes one bleeding before the turkey hens went into egg production, five throughout the laying season, and one bleeding at the termination of egg production and after the turkey hens were placed on range with access to green grass in addition to their regular ration.

Five turkey hens out of 25 hens were selected at random from each of the four pens for bleeding. Blood samples were taken from the 20 hens at four-week intervals prior to and throughout the laying season. One turkey tom from each pen was bled each time. The blood samples were collected from the Vena humeri profunda of the wing with a syringe and

needle. One cc. of saturated sodium citrate was added for each 50 cc. of blood as an anticoagulant.

The rations fed the four different groups are shown in the following tables

Table No. I

	GROUPS I II	GROUPS III IV
	(percent)	(percent)
Mash		
Yellow corn	25	25
Wheat bran	15	15
Wheat middlings	14	14
Minerals:		
Meat and bone scraps	15	15
Soybean oil meal	10	10
Alfalfa leaf meal	5	5
Dried buttermilk	10	10
Salt mixture *	2	2
Ground limestone	3.5	3.5
Fish oil concentrate 400 I. U. vit. D and 3000 I. U. vit. A	.5	.5
Scratch grains	sprouted oats corn oats	corn oats

* Mn and I included

(oyster shells, grit, and water were provided ad libitum.)

Groups I and II had the same rations except the mash fed to Group II was pelleted while Group I received non-pelleted mash which was made up fresh every two weeks. Groups III and IV had the same rations with pelleted mash fed to Group IV and non-pelleted mash to Group III. Sufficient pelleted mash was prepared at the start of the experiment to carry Groups II and IV through the entire period.

Workers at the Pennsylvania Agricultural Experiment Station (1943) have shown that the hatchability of turkey eggs is appreciably influenced by riboflavin intake whereas

egg production, body weight, mortality, and fertility are not measurably influenced by riboflavin intake. The minimum level for riboflavin intake was reported to be 1875 micrograms per pound of feed with a hatchability record of 78 percent. An assay of the mash used in the 1943 study showed the riboflavin content to be 1400 micrograms per pound of mash. The hatchability was 24 percent for this study. In 1944-45, a similar experiment was conducted at this Station and the riboflavin content of the mash was increased from 1400 to 1900 micrograms per pound of mash. The rations used in this experiment were comprised of mash plus scratch grains whereas in 1944-45 the turkeys were fed an all-mash breeder ration. Therefore, the increase in riboflavin intake would be much greater than 500 micrograms and would approximate 100 percent. The hatchability went from 24 to 78 percent. This would indicate that there was a riboflavin deficiency for maximum hatchability and the true relationship between hatchability and the blood picture may not have been revealed.

The averages for the egg production, fertility, and hatchability records are presented in the following table.

Table II

Group No.	Hen No.	Egg Production (number)	Fertility (percent)	Hatchability* (percent)
I	1607	85	71	6
I	1612	72	63	12
I	1619	71	88	33
I	1626	107	71	27
I	1628	7	43	0
Average		68	72	20
II	1633	78	100	29
II	1644	79	94	22
II	1648	67	58	5
II	1649	83	69	16
II	1655	79	97	5
Average		77	84	16
III	1659	95	96	59
III	1667	90	98	39
III	1670	88	99	6
III	1675	80	97	28
III	1678	78	89	28
Average		86	96	33
IV	1693	72	88	14
IV	1698	1	100	0
IV	1703	44	81	60
IV	1708	17	93	0
IV	1709	58	100	15
Average		38	90	22

* Percent hatchability of fertile eggs.

The number of eggs laid by individual turkey hens between bleeding dates is summarized in the following table.

Table III

Group No.	Hen No.	Period between Bleeding Dates								Totals
		12-28	12-28 to 1-25	1-25 to 2-22	2-22 to 3-21	3-21 to 4-18	4-18 to 5-16	5-16 to 5-29	5-29 until sold	
I	1607	0	1	19	12	20	20	6	7	85
I	1612	0	0	4	22	18	19	5	4	72
I	1619	0	0	8	17	15	10	11	10	71
I	1626	0	0	23	26	23	18	8	9	107
I	1628	0	0	4	3	0	0	0	0	7
Totals		0	1	58	80	76	67	30	30	342
II	1633	0	0	9	18	20	14	8	9	78
II	1644	0	0	18	23	18	11	6	3	79
II	1648	0	0	5	16	17	19	8	2	67
II	1649	0	0	17	18	18	15	7	8	83
II	1655	0	0	15	20	22	15	6	1	79
Totals		0	0	64	95	95	74	35	23	386
III	1659	0	0	10	22	23	19	11	10	95
III	1667	0	0	14	19	20	21	8	8	90
III	1670	0	0	15	19	21	21	5	7	88
III	1675	0	0	17	14	21	20	6	2	80
III	1678	0	0	19	20	18	17	2	2	78
Totals		0	0	75	94	103	98	32	29	451
IV	1693	0	0	12	17	17	12	8	6	72
IV	1698	0	0	1	0	0	0	0	0	1
IV	1703	0	0	5	16	5	9	4	7	44
IV	1708	0	0	2	15	0	0	0	0	17
IV	1709	0	0	12	8	13	10	5	10	58
Totals		0	0	32	56	33	31	17	23	192
Total all Groups		0	1	229	325	307	270	114	105	1351

The total number of eggs produced has been shown for individual birds because it has a very definite effect on the blood picture as will be shown.

Methods:

Hemoglobin:

Hemoglobin was determined by the Evelyn method (1936). The details of this procedure are as follows: Dilute .02 cc. of fresh, citrated, whole blood in 5 cc. of water. Add 5 cc. of 0.8 percent NH_4OH, mix well, and read immediately in the photoelectric colorimeter with the 520 mu filter, using water for a blank. Grams of hemoglobin per 100 cc. blood equals (2 - Log G) (38.8).

Calcium:

Calcium was determined on the plasma using the standard titration method of Clark and Collip (1925).

Seven cc. of water and 1 cc. of saturated ammonium oxalate are pipetted into a 15 ml. conical centrifuge tube and 2 cc. of plasma added. Mix by inversion. Allow to stand over night at room temperature. Centrifuge for 15 minutes or longer at 2500 r.p.m. Decant the supernatant liquid. Wash three times with 3 cc. portions of 2.5 percent NH_4OH. Dissolve the calcium oxalate in 3 cc. of approximately 1 N sulphuric acid. Heat to 80° - 90° C. and titrate with standard 0.01 N. $KMnO_4$. One cc. of 0.01 N $KMnO_4$ = 0.2004 mgm. of Ca.

Inorganic phosphorus:

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Inorganic phosphorus:

Inorganic phosphorus was determined on plasma according

to the Fiske and Subbarow method (1925) modified for use with the Evelyn colorimeter.

Reagents:

1. 10 o/o trichloroacetic acid.

2. Molybdate I: 25 gms. of ammonium molybdate, 500 cc. of 10 N sulphuric acid, to a liter with water. This solution is used in preparing the calibration curve.

3. Molybdate II: 25 gms. of ammonium molybdate, 300 cc. of 10 N sulphuric acid, to a liter with water.

4. Phosphorus reagent: 14.25 gms. of sodium bisulfite, 0.25 gms. of 1-amino-2-naphthol-4-sulfonic acid (Eastman Kodak purified), 80 cc. of water, 10 cc. of 5 o/o anhydrous sodium sulfite. Shake and add more sodium sulfite if necessary for solution. Dilute to 100 cc. with water.

   The reagent is ready for use as soon as solution is complete, and may be used for at least a week.

Procedure:

The plasma proteins are precipitated by accurately pipetting 0.5 cc. of plasma into 9.5 cc. of 10 percent trichloroacetic acid. Mix by inversion. Let stand until precipitate starts to settle out. Centrifuge for 15 minutes at 2000 r.p.m. Pipette off 5 ml. of the protein-free filtrate into a colorimeter tube. This will contain 0.25 cc. of plasma. Make a blank containing 5 ml. of 10 percent trichloroacetic acid and treat in same manner as unknowns. Add in the sequence given: 1 ml. of molybdate II solution, 0.4 ml. of phosphorus reagent, and make up to 10 cc. with

water. Let stand 10 minutes and read in the photoelectric colorimeter with the 660 mu filter. The blank is set so G = 100 and a zero or center setting obtained.

Calibration and calculation:

A stock standard solution is prepared by dissolving 4.39 grams of potassium dihydrogen phosphate in a liter of water containing 10 cc. of 10 N sulphuric acid. This would contain 1 mgm. of phosphorus per cc.

From this stock standard, dilute solutions are made containing, in a volume of 5 cc., amounts of phosphorus varying from 0.0025 to 0.03 mgm. (corresponding to phosphorus concentrations of 1.0 to 12.0 mgm. per 100 cc. of plasma).

The standard sample tubes are made up by pipetting into colorimeter tubes 5 cc. of the dilute phosphorus standard solutions, 1 cc. of molybdate I, 0.4 cc. of the phosphorus reagent, made up to 10 cc. with water. The blank contains 8.6 cc. water, 1 cc. of molybdate I, and 0.4 cc. of the phosphorus reagent. Let stand 10 minutes and read with 660 mu filter.

If $X = 0.020$ mgm. of phosphorus
and $L = .465$

Then

$$K_1 = \frac{X}{L} = \frac{0.020}{.465} = 0.0430$$

The unknown tubes contain 0.25 cc. of plasma, so

$$K = K_1 \frac{100}{0.25} = \frac{(0.0430)\ (100)}{(0.25)} = 17.20$$

Therefore: $X = KL = 17.20\ L$

Carotene* and vitamin A:

The method used for carotene and vitamin A in plasma is a modification of the Kimble method (1939) involving the Carr-Price reaction.

Carotene:

The carotene and vitamin A of plasma are extracted in petroleum ether. The plasma proteins are precipitated with alcohol, and pigments soluble in alcohol and insoluble in ether; such as, bile pigments, are dissolved in the alcohol.

The extraction is accomplished by accurately measuring 13 cc. of petroleum ether, 3.5 to 5 cc. of plasma, and 5 cc. of 95 percent alcohol into a 25 ml., glass stoppered, conical centrifuge tube. A good seal can be made if a small drop of mineral oil is put on the upper part of the glass stopper. This prevents any loss of ether when the tubes are inverted for mixing and while centrifuging. Precaution should be taken so that the mineral oil is confined to the ground glass portion of the tube. If any of the oil should get into the colorimeter tube, it will cause a cloudiness in the vitamin A determination.

* The yellow pigment extracted by petroleum ether is referred to as carotene in this paper but would include all the carotenoids.

Sufficient mixing to insure complete precipitation of plasma proteins and extractions of carotene and vitamin A can be accomplished by an end-over-end inversion of the tubes for 10 minutes. One must refrain from shaking the tubes to prevent the breaking down of the precipitated proteins which might hinder the phasic separation of alcohol and ether. The tubes are then centrifuged at 2000 r.p.m. for 15 minutes.

A 10 ml. portion of the ether layer is pipetted into a colorimeter tube. Precaution must be taken to pipette off only petroleum ether as alcohol will cause cloudiness when the vitamin A is taken up in antimony trichloride.

Read in a photoelectric colorimeter using a 440 mu filter. Use petroleum ether for a blank. Record the galvanometer reading as G_1. Look up L_1 value on table where $L = 2 - \log G_1$. When 5 cc. plasma are used and β-carotene is used in making the calibration curve, K = 0.676:

when $X = 65\ \gamma$ β-carotene (crystalline)
$L_1 = 0.25$

$$K_{\text{Pet. soln}} = \frac{X}{L_1} = \frac{65}{0.25} = 2.60$$ (results in terms of γ β-carotene per cc.)

$$K_{\text{Plasma}} = K_{\text{Pet. soln.}} \times \frac{13}{5} \times 100 = 2.60 \times \frac{13}{5} \times 100 = 676\ \gamma\ \beta\text{-carotene/100 cc.}$$

$\frac{676}{1000} L_1$ = mgm. of β-carotene /100 cc. plasma

Vitamin A

Using the 10 ml. of petroleum ether solution from the carotene determination, the petroleum ether is evaporated off. This can be conveniently done by placing the colorimeter tubes on warm sand, about 45°-50° C. If the sand is in a shallow pan, the tubes can be laid on top of the sand at about a 30 degree angle and the ether evaporates without overheating. It is a good idea to turn the tubes frequently until evaporation starts, to prevent bumping. If the sand is too warm, bumping may cause the loss of ether by boiling out of the tube and may destroy vitamin A. When the ether is practically evaporated, remove from sand bath and shake to dryness. Every precaution should be taken to avoid overheating during evaporation.

The tubes are rinsed by dipping in hot water and dried to remove any sand that might adhere to the tubes. Care should be taken to remove the sand particles without scratching the colorimeter tubes. The residue is taken up in 1 ml. of chloroform which is moisture-free. If after the addition of the 25 percent $SbCl_3$ reagent the solution is cloudy, it may be due to the presence of moisture in either the reagent or the chloroform. This can usually be cleared up by the addition of one drop of acetic anhydride to the chloroform extract prior to the addition of the $SbCl_3$ reagent. This one drop of acetic

anhydride does not interfere with the Carr Price reaction. Duplicate samples were run where one tube contained one drop of acetic anhydride while one tube had none added and checks were obtained consistently. An antimony trichloride reagent blank is set at 100 in the colorimeter with filter 620 mu, the center or zero setting determined and allowed to become absolutely stable. The unknown tube containing the chloroform extract is then put in place in the colorimeter and 9 cc. of $SbCl_3$ reagent added from a rapid delivery pipette or automatic burette such as described by Oser, Melnick, and Pader (1943). Calculations involving correction for carotene were made according to Koehn and Sherman (1940). The factor used to convert I. U. of vitamin A to micrograms of vitamin A was 0.25.

RESULTS AND DISCUSSION

Tables 1-5 give the mean, range, and the number of analyses by groups and dates for hemoglobin, calcium, inorganic phosphorus, carotene, and vitamin A for the turkey hens. Table 6 summarizes all of the blood data for the turkey toms.

Table No. 1

HEMOGLOBIN CONTENT OF TURKEY BLOOD AT SEVERAL DIFFERENT DATES

Group No.	Date	No. of analyses	Mean	Range
			(percent)	(percent)
I	December 28, 1943	5	13.0	12.0-13.8
II	December 28, 1943	5	13.2	12.2-14.6
III	December 28, 1943	5	12.9	11.6-14.0
IV	December 28, 1943	5	13.6	12.4-14.6
All groups		20	13.2	11.6-14.6
I	January 25, 1944	5	13.4	11.6-14.7
II	January 25, 1944	5	12.4	12.0-12.8
III	January 25, 1944	5	12.6	11.2-13.8
IV	January 25, 1944	5	12.5	11.8-13.6
All groups		20	12.7	11.2-14.7
I	February 22, 1944	5	10.2	9.4-11.5
II	February 22, 1944	5	10.9	10.2-11.5
III	February 22, 1944	5	10.6	9.2-12.6
IV	February 22, 1944	5	11.0	9.6-12.2
All groups		20	10.6	9.2-12.6
I	March 25, 1944	5	10.8	9.9-11.8
II	March 25, 1944	5	10.4	9.7-11.8
III	March 25, 1944	5	10.6	9.0-12.2
IV	March 25, 1944	5	11.0	8.6-12.0
All groups		20	10.7	8.6-12.2
I	April 18, 1944	5	9.7	8.9-10.2
II	April 18, 1944	5	9.1	8.3-10.6
III	April 18, 1944	5	9.7	9.0-10.3
IV	April 18, 1944	5	11.3	9.5-13.4
All groups		20	9.9	8.3-13.4
I	May 16, 1944	5	9.1	7.6-9.9
II	May 16, 1944	5	9.6	8.7-10.3
III	May 16, 1944	5	9.1	8.2-10.4
IV	May 16, 1944	5	10.9	9.6-13.0
All groups		20	9.7	7.6-13.0
I	May 29, 1944	5	9.5	8.2-10.6
II	May 29, 1944	5	9.6	9.2-10.0
III	May 29, 1944	5	9.6	9.0-10.6
IV	May 29, 1944	5	9.8	8.1-12.2
All groups		20	9.6	8.1-12.2

* From the South Dakota State College Poultry Department flock.

Table No. 2

CALCIUM CONTENT OF TURKEY* BLOOD AT SEVERAL DIFFERENT DATES

Group No.	Date	No. of analyses	Mean	Range
			mgm/100 cc.	mgm/100 cc.
I	December 28, 1943	5	9.20	8.42-9.69
II	December 28, 1943	5	9.27	7.90-11.37
III	December 28, 1943	5	9.50	9.06-10.32
IV	December 28, 1943	5	9.03	7.79-10.95
All groups		20	9.25	7.79-11.37
I	January 25, 1944	5	23.00	8.58-38.28
II	January 25, 1944	5	18.24	14.74-21.06
III	January 25, 1944	5	21.01	14.26-25.85
IV	January 25, 1944	5	15.61	9.22-21.38
All groups		20	19.46	8.58-38.28
I	February 22, 1944	5	29.07	15.81-43.18
II	February 22, 1944	5	25.36	21.69-28.48
III	February 22, 1944	5	25.97	20.88-30.20
IV	February 22, 1944	5	21.51	9.93-26.96
All groups		20	25.47	9.93-43.18
I	March 25, 1944	5	20.25	9.56-26.04
II	March 25, 1944	5	26.23	23.01-30.92
III	March 25, 1944	5	26.90	16.92-34.46
IV	March 25, 1944	5	13.20	7.75-23.92
All groups		20	21.64	7.75-34.46
I	April 18, 1944	5	26.71	16.92-34.96
II	April 18, 1944	5	25.74	24.02-28.38
III	April 18, 1944	5	26.04	21.58-30.20
IV	April 18, 1944	5	19.31	10.94-33.44
All groups		20	24.45	10.94-34.96
I	May 16, 1944	5	23.43	17.13-30.30
II	May 16, 1944	5	25.51	21.94-28.28
III	May 16, 1944	5	30.30	23.31-36.89
IV	May 16, 1944	5	21.54	13.78-33.64
All groups		20	25.19	13.78-36.89
I	May 29, 1944	5	20.96	10.54-30.10
II	May 29, 1944	5	21.08	14.19-23.82
III	May 29, 1944	5	20.72	13.58-26.86
IV	May 29, 1944	5	18.30	6.69-25.24
All groups		20	20.26	6.69-30.10

* From the South Dakota State College Poultry Department flock.

Table No. 3

INORGANIC PHOSPHORUS CONTENT OF TURKEY BLOOD AT SEVERAL DIFFERENT DATES*

Group No.	Date	No. of analyses	Mean	Range
			mgm/100 cc.	mgm/100 cc.
I	December 28, 1943	5	4.77	4.06-5.18
II	December 28, 1943	5	4.40	3.77-5.64
III	December 28, 1943	5	4.15	3.75-4.76
IV	December 28, 1943	5	4.31	3.19-5.38
All groups		20	4.41	3.19-5.64
I	January 25, 1944	5	5.56	2.48-9.38
II	January 25, 1944	5	4.86	3.24-6.29
III	January 25, 1944	5	5.85	4.43-7.08
IV	January 25, 1944	5	5.34	4.26-5.86
All groups		20	5.40	2.48-9.38
I	February 22, 1944	5	6.95	4.23-8.44
II	February 22, 1944	5	5.80	3.77-6.91
III	February 22, 1944	5	6.63	4.52-8.57
IV	February 22, 1944	5	6.23	3.83-8.91
All groups		20	6.40	3.77-8.91
I	March 25, 1944	5	5.83	4.83-7.48
II	March 25, 1944	5	5.98	4.87-7.48
III	March 25, 1944	5	6.83	3.75-9.75
IV	March 25, 1944	5	4.28	3.22-6.36
All groups		20	5.73	3.22-9.75
I	April 18, 1944	5	6.27	4.08-8.04
II	April 18, 1944	5	7.19	6.70-7.53
III	April 18, 1944	5	7.45	5.26-9.68
IV	April 18, 1944	5	6.25	3.66-9.48
All groups		20	6.79	3.66-9.68
I	May 16, 1944	5	7.18	5.27-9.41
II	May 16, 1944	5	7.49	6.47-9.00
III	May 16, 1944	5	7.39	6.24-8.35
IV	May 16, 1944	5	6.30	4.88-7.48
All groups		20	7.09	4.88-9.41
I	May 29, 1944	5	6.08	5.16-6.91
II	May 29, 1944	5	6.82	5.74-7.40
III	May 29, 1944	5	6.65	4.33-8.88
IV	May 29, 1944	5	6.87	4.92-8.88
All groups		20	6.60	4.33-8.88

* From the South Dakota State College Poultry Department flock.

Table No. 4

CAROTENE CONTENT OF TURKEY* BLOOD AT SEVERAL DIFFERENT DATES

Group No.	Date	No. of analyses	Mean	Range
			mgm/100 cc.	mgm/100 cc.
I	December 28, 1943	5	.2923	.2261-.4101
II	December 28, 1943	5	.2999	.1869-.4642
III	December 28, 1943	5	.2712	.2378-.3414
IV	December 28, 1943	5	.3694	.2499-.5430
All groups		20	.3082	.1369-.5430
I	January 25, 1944	5	.2224	.1000-.3966
II	January 25, 1944	5	.2004	.0897-.4078
III	January 25, 1944	5	.1779	.0710-.3223
IV	January 25, 1944	5	.2769	.1200-.3572
All groups		20	.2194	.0710-.4078
I	February 22, 1944	5	.1179	.0543-.2090
II	February 22, 1944	5	.0694	.0277-.0838
III	February 22, 1944	5	.0788	.0543-.1279
IV	February 22, 1944	5	.0709	.0569-.0766
All groups		20	.0842	.0277-.2090
I	March 21, 1944	5	.1080	.0710-.1728
II	March 21, 1944	5	.1070	.0475-.1608
III	March 21, 1944	5	.1306	.0911-.1591
IV	March 21, 1944	5	.1017	.0584-.1376
All groups		20	.1118	.0475-.1728
I	April 18, 1944	5	.1260	.0618-.2143
II	April 18, 1944	5	.0867	.0538-.1715
III	April 18, 1944	5	.1100	.0758-.1586
IV	April 18, 1944	5	.1047	.0715-.2035
All groups		20	.1068	.0538-.2143
I	May 16, 1944	5	.1008	.0480-.1469
II	May 16, 1944	5	.0840	.0524-.1376
III	May 16, 1944	5	.1614	.0853-.3802
IV	May 16, 1944	5	.1274	.0561-.2285
All groups		20	.1184	.0480-.3802
I	May 29, 1944	5	.3908	.1624-.5719
II	May 29, 1944	5	.3586	.2346-.5286
III	May 29, 1944	5	.3685	.1850-.6550
IV	May 29, 1944	5	.4549	.2548-.6834
All groups		20	.3932	.1624-.6834

* From the South Dakota State College Poultry Department flock.

Table No. 5

VITAMIN A CONTENT OF TURKEY* BLOOD AT SEVERAL DIFFERENT DATES

Group No.	Date	No. of analyses	Mean mgm/100 cc.	Range mgm/100 cc.
I	December 28, 1943	5	.0966	.0815-.1188
II	December 28, 1943	5	.0932	.0763-.1245
III	December 28, 1943	5	.1104	.0870-.1298
IV	December 28, 1943	5	.1099	.0805-.1345
All groups		20	.1025	.0763-.1345
I	January 25, 1944	5	.0911	.0658-.1127
II	January 25, 1944	5	.0645	.0446-.0842
III	January 25, 1944	5	.0869	.0671-.1066
IV	January 25, 1944	5	.1005	.0734-.1193
All groups		20	.0858	.0446-.1193
I	February 22, 1944	5	.0782	.0387-.1168
II	February 22, 1944	5	.0597	.0539-.0687
III	February 22, 1944	5	.0670	.0427-.0807
IV	February 22, 1944	5	.0783	.0421-.1597
All groups		20	.0708	.0387-.1597
I	March 21, 1944	5	.0585	.0333-.0836
II	March 21, 1944	5	.0418	.0192-.0563
III	March 21, 1944	5	.0787	.0602-.0903
IV	March 21, 1944	5	.0716	.0362-.1282
All groups		20	.0627	.0192-.1282
I	April 18, 1944	5	.0577	.0192-.0837
II	April 18, 1944	5	.0263	.0173-.0421
III	April 18, 1944	5	.0568	.0414-.0808
IV	April 18, 1944	5	.0586	.0258-.1282
All groups		20	.0499	.0173-.1282
I	May 16, 1944	5	.0585	.0354-.0957
II	May 16, 1944	5	.0258	.0142-.0477
III	May 16, 1944	5	.0617	.0302-.1172
IV	May 16, 1944	5	.0726	.0495-.0947
All groups		20	.0546	.0142-.1172
I	May 29, 1944	5	.1023	.0449-.1298
II	May 29, 1944	5	.0944	.0646-.1268
III	May 29, 1944	5	.0991	.0652-.1275
IV	May 29, 1944	5	.1257	.1083-.1356
All groups		20	.1054	.0449-.1356

*From the South Dakota State College Poultry Department flock.

Table No.6

SOME BLOOD LEVELS FOR TURKEY TOMS [1] AT SEVERAL DIFFERENT DATES

Group No.	Date	Hemo-globin	Ing. [2] P	Ca [3]	Carotene	Vit. A
		percent	mgm/100 cc.			
I	Dec. 28, 1943	12.8	4.58	11.90	.4608	.0818
II	Dec. 28, 1943	14.4	3.52	9.79	.3200	.0921
III	Dec. 28, 1943	15.2	3.12	10.64	.3223	.0896
IV	Dec. 28, 1943	13.5	3.92	9.58	.4968	.1228
All groups		14.0	3.78	10.48	.4000	.0966
I	Jan. 25, 1944	14.4	4.28	7.95	.5464	.0902
II	Jan. 25, 1944	12.8	4.14	7.00	.4574	.1110
III	Jan. 25, 1944	14.0	4.22	9.16	.5352	.0797
IV	Jan. 25, 1944	13.7	4.54	8.26	.5937	.1309
All groups		13.7	4.30	8.09	.5332	.1030
I	Feb. 22, 1944	13.2	3.65	8.72	.4416	.0788
II	Feb. 22, 1944	12.9	3.82	8.31	.2645	.0954
III	Feb. 22, 1944	13.8	2.74	8.82	.6061	.0567
IV	Feb. 22, 1944	13.4	4.26	9.93	.4833	.0965
All groups		13.3	3.62	8.94	.4489	.0818
I	March 25, 1944	14.3	3.60	7.50	.4101	.0691
II	March 25, 1944	13.9	2.95	7.80	.3797	.0590
III	March 25, 1944	12.9	2.55	6.59	.1625	.0589
IV	March 25, 1944	14.9	3.76	8.01	.4799	.0921
All groups		14.0	3.22	7.48	.3580	.0698
I	April 18, 1944	12.4	3.51	10.34	.2819	.0595
II	April 18, 1944	12.6	4.13	10.34	.2859	.0539
III	April 18, 1944	12.6	3.14	10.24	.1850	.0494
IV	April 18, 1944	----	----	----	----	----
All groups		12.5	3.59	10.31	.2509	.0543
I	May 16, 1944	13.2	4.18	13.48	.1553	.0709
II	May 16, 1944	13.4	4.12	9.02	.2382	.0622
III	May 16, 1944	13.4	3.48	8.41	.3059	.0942
IV	May 16, 1944	----	----	----	----	----
All groups		13.3	3.93	10.30	.2331	.0758
I	May 29, 1944	12.3	8.22	5.78	.1254	.0667
II	May 29, 1944	12.3	4.28	6.99	.3515	.0822
III	May 29, 1944	12.8	4.18	7.90	.2346	.0853
IV	May 29, 1944	----	----	----	----	----
All groups		12.5	5.56	6.89	.2372	.0781

1/ From the South Dakota College Poultry Department flock.

2/ P = Phosphorus

3/ Ca = Calcium

An analysis of the variance of the data for the turkey hens showed that there were highly significant differences in hemoglobin, calcium, and vitamin A levels between the four different groups. Also, it showed that there were highly significant differences in hemoglobin, calcium, inorganic phosphorus, carotene, and vitamin A levels between the seven different dates. However, the differences for these various blood constituents between groups times dates were not significant. Table 7 gives the sources of variation, degrees of freedom, and mean squares for this analysis.

Table 7 — Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square or Variance				
		Hb	Ca	P	Carotene	Vit. A
Groups	3	13 xx	248 xx	3.7	.012	.0056 xx
Dates	6	46 xx	636 xx	18 xx	.28 xx	.0099 xx
Dates X Groups	18	0.30	26.9	1.4	.0055	.0003
Experimental Error	112	0.98	55.3	2.1	.0081	.0005

xx F values (Snedecor, 1937) exceeds 1 percent level of significance.

The egg production record, Table III, shows considerable variation in the average number of eggs laid per group. Group I had an average egg production of 68 eggs; Group II, 77 eggs; Group III, 86 eggs; and Group IV, 38 eggs; with an average for all groups of 67 eggs. This compares favorably with the egg production for Broad-Breasted Bronze turkey hens of 57 eggs

per turkey hen in 1941 and 60 eggs per turkey hen in 1942 reported by Whitson, Marsden, and Titus (1944). Therefore, the egg production for the turkeys used in this study is considered to be normal.

The difference in number of eggs produced by the four different groups may account for the highly significant differences between groups for hemoglobin, calcium, and vitamin A levels. The number of hens per group is insufficient for testing the two different rations fed. The rations used in this experiment should have been adequate in calcium, phosphorus, iron, carotene, and vitamin A for breeding stock (Titus, 1959).

There were highly significant differences for the five blood constituent levels between dates, indicating that changes in hemoglobin, calcium, inorganic phosphorus, carotene, and vitamin A accompanies egg production. On the first bleeding date (December 28) none of the hens were laying but the hens were all in egg production by the third bleeding date (February 22).

Since there was evidence that a change in the blood picture accompanies egg production the correlation coefficients were calculated for egg production versus each of the five blood constituents studied.

Table 8 gives the correlation coefficients, the standard deviation of the correlation coefficients, and the t values used in testing the significance of the correlation coefficients.

Table 8. — Table of Correlation Coefficients

Variables Correlated	r [1]	σ_r [2]	t [3]
Hemoglobin-Calcium	-0.676	0.0544	12.4 xx
Hemoglobin-Phosphorus	-0.707	0.0423	16.7 xx
Calcium-Phosphorus	0.735	0.0389	18.9 xx
Carotene-Vitamin A	0.701	0.0430	16.3 xx
Hemoglobin-Egg Production [4]	-0.637	0.0542	11.8 xx
Calcium-Egg Production [5]	0.484	0.0648	7.47 xx
Phosphorus-Egg Production [4]	0.449	0.0729	6.16 xx
Carotene-Egg Production [4]	-0.554	0.0633	8.75 xx
Vitamin A-Egg Production [4]	-0.507	0.0679	7.47 xx
Fertility-Egg Production [6]	0.080	0.0801	1.00

1/ r = Correlation coefficient
2/ σ_r = Standard deviation of correlation coefficient
3/ $t = \frac{r}{\sigma_r}$
4/ Nine day egg producing period. Four days before and after the bleeding date plus the day of bleeding.
5/ Five day egg producing period. Two days before and after the bleeding date plus the day of bleeding.
6/ Fourteen day period. Includes all eggs produced between incubation periods.

The last bleeding date (May 29) was not included in the calculation of these correlation coefficients because the turkey hens were placed on range with access to green grass in addition to their regular rations. Also, the egg production after May 15 was not representative of either a

laying hen or a non-laying hen.

The negative correlations between egg production and hemoglobin, carotene, and vitamin A were highly significant (odds greater than 99:1). These negative relations are apparent in Figs. 1, 4, and 5 by a decrease in the hemoglobin, carotene, and vitamin A levels with advancement into the egg production period.

The positive correlations between egg production and calcium and inorganic phosphorus are also highly significant. These positive relations are apparent in Figs. 2 and 3 by an increase in the calcium and inorganic phosphorus levels with advancement into the egg production period.

There was negative correlation between hemoglobin and calcium and between hemoglobin and inorganic phosphorus, however, the correlation between calcium and inorganic phosphorus and between carotene and vitamin A was positive. This is also noted in Fig. 6 when mean values for hemoglobin, calcium, inorganic phosphorus, carotene, and vitamin A were plotted against bleeding dates. With advancement into the egg production period, hemoglobin shows a downward trend while calcium and inorganic phosphorus show upward trends. Carotene and vitamin A both show downward trends with advancement into the egg production period.

In Table 8 it was shown that there was no appreciable correlation between fertility and egg production. Whitson et al. (1944) have reported similar results. The average percentage fertility of eggs set was 72, 84, 96, and 90

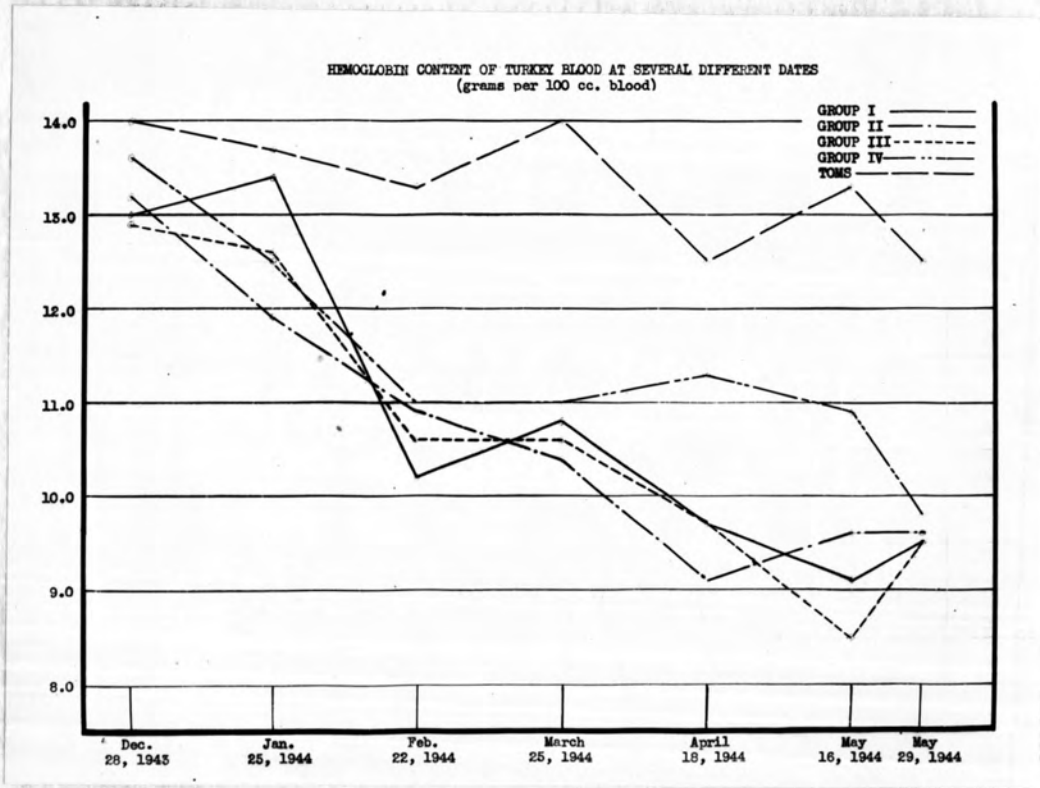

Fig. 1 Average hemoglobin values for turkey hens and toms.

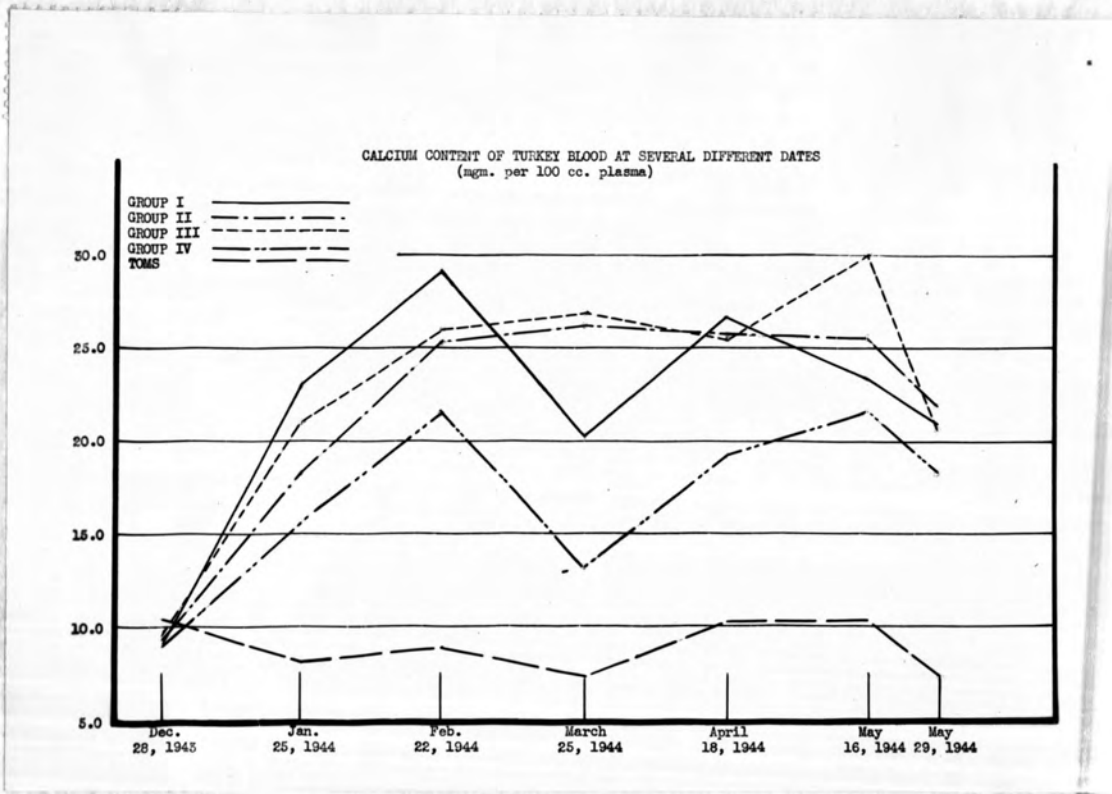

Fig. 2 Average plasma calcium values for turkey hens and toms.

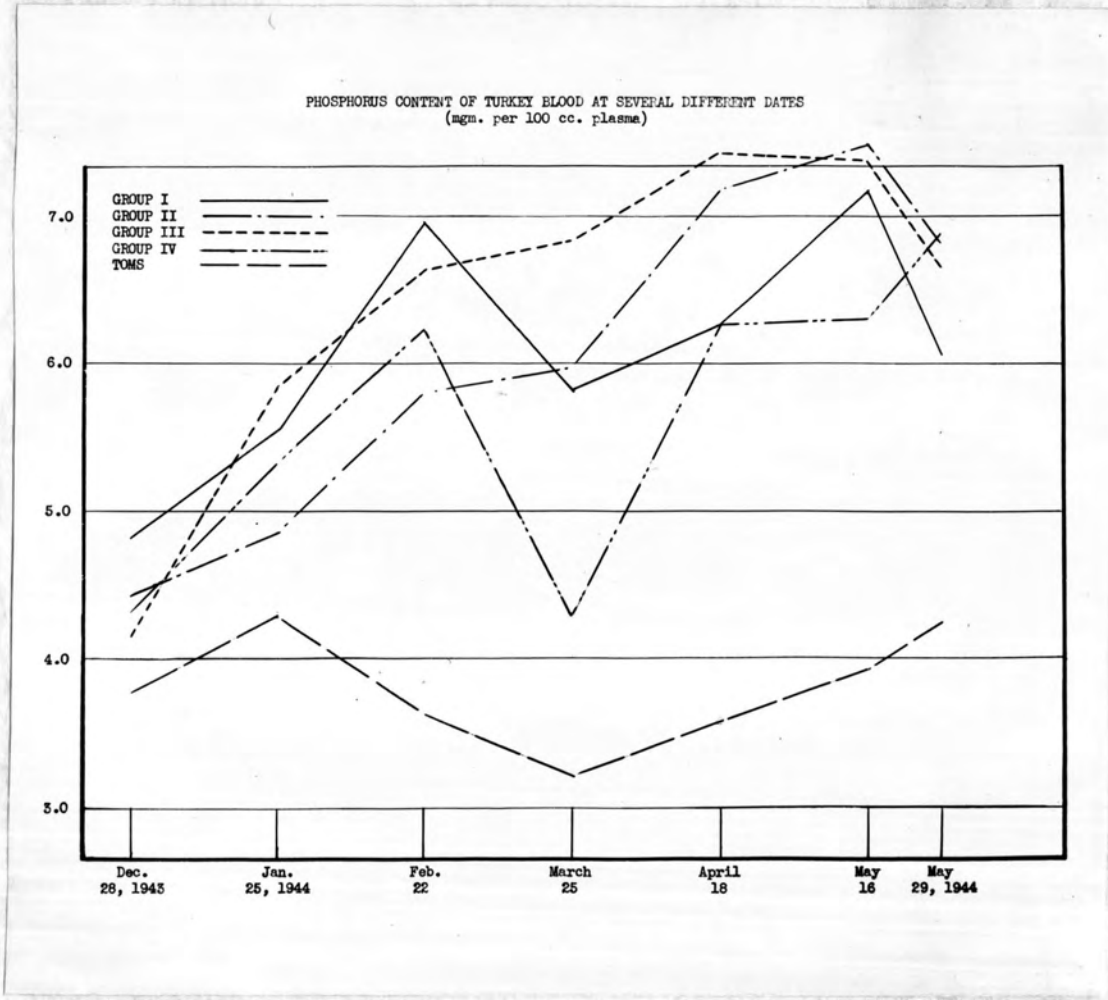

Fig. 3 Average inorganic phosphorus values for turkey hens and toms.

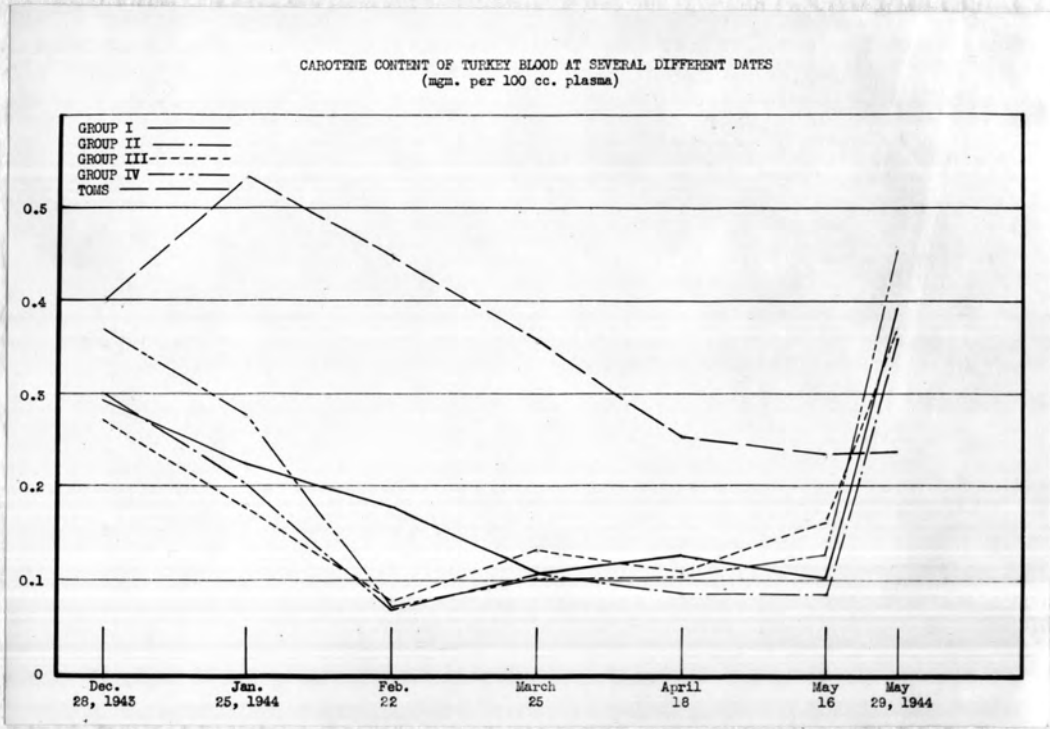

Fig. 4 Average carotene values for turkey hens and toms.

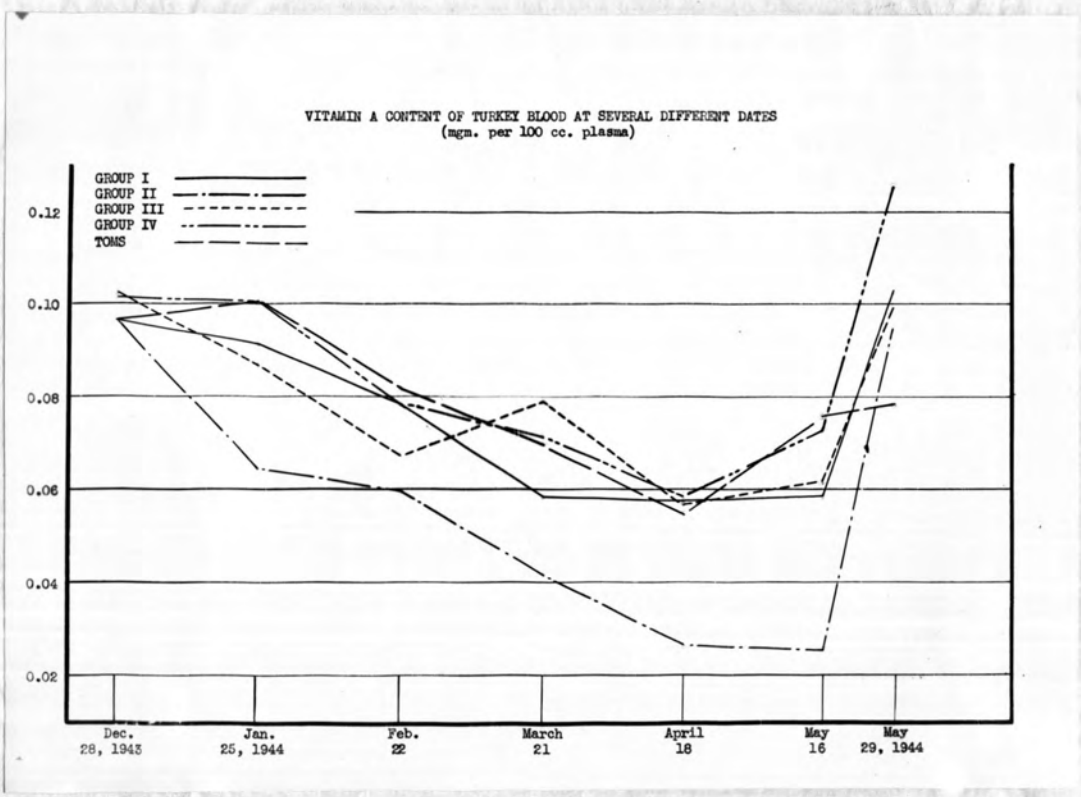

Fig. 5 Average vitamin A values for turkey hens and toms.

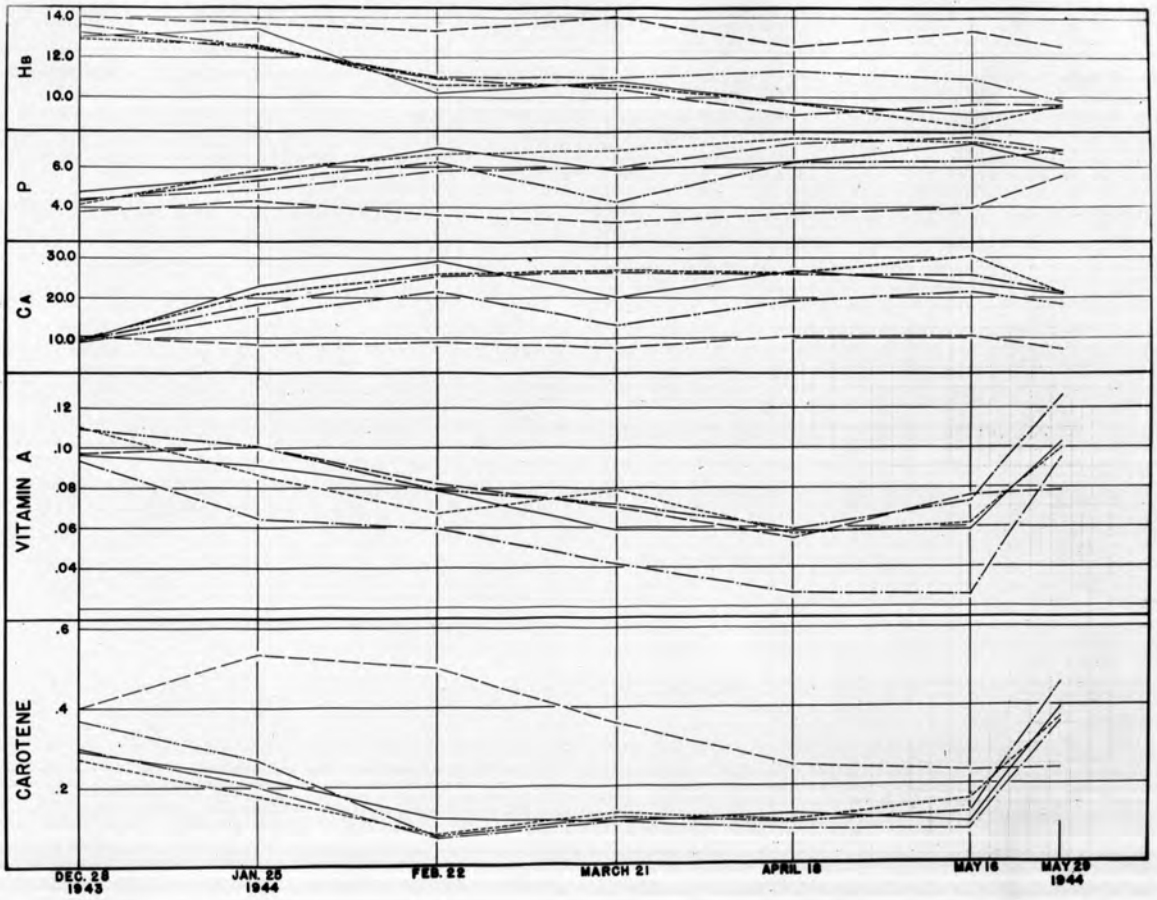

Fig. 6 Graphic summary of all the turkey blood data.

Legend

Hens:

Group I ——————————————————————
Group II ——— – ——— – ——— – ——— – ———
Group III – – – – – – – – – – – – – – – – –
Group IV ——— – – ——— – – ——— – – ——— – –

Toms: ——— ——— ——— ——— ——— ——— ——— ——— ———

Hb ..... gms. per 100 cc. blood
P ..... mgms. per 100 cc. plasma
Ca ..... mgms. per 100 cc. plasma
Vitamin A ..... mgms. per 100 cc. plasma
Carotene ..... mgms. per 100 cc. plasma

for Groups I, II, III, and IV respectively. The average percentage fertility for all groups was 86. This compares favorably with the values of 76 and 88 percent fertility of eggs from Broad-Breasted Bronze turkeys reported by Whitson et al. (1944). Milby and Thompson (1945) report similar values for fertility of eggs for Broad-Breasted Bronze turkeys. The percentage fertility of eggs set is considered to be average for the turkeys used in this study. Highly significant correlation was found between egg production and hemoglobin, calcium, inorganic phosphorus, carotene, and vitamin A, but there was no appreciable correlation between egg production and fertility of the eggs set. Consequently, one would not expect any appreciable correlation between fertility of the eggs and concentration of hemoglobin, calcium, inorganic phosphorus, carotene, and vitamin A in the blood.

The percentage hatchability of fertile eggs was 20, 16, 33, and 22 for Groups I, II, III, and IV respectively. The average percentage hatchability of fertile eggs for all groups was 24. This is about fifty percent of the normal percentage hatchability as reported by Whitson et al. (1944) and about one-third the percent hatchability of 78 percent reported by Pennsylvania Agricultural Experiment Station (1945) and South Dakota Agricultural Experiment Station*. It has been suggested that the low hatchability was due to riboflavin deficiency. Since the hatchability record shown in Table II was abnormal any relationship of hatchability

* South Dakota Agricultural Experiment Station Annual Report. 1944-45. In Press.

to the blood picture may have been masked. The normal egg production, fertility, and the fact that the turkeys received concentrated vitamin A in their ration which was considered adequate for breeding stock are further evidences that it was perhaps a deficiency of a vitamin other than vitamin A. It is assumed that the hatchability would not effect the values of the blood constituents studied and the turkeys are considered to be normal.

The Broad-Breasted Bronze turkey hens used in this study were free from disease, showed no dietary deficiency symptoms, housing facilities were desirable, and the hens were properly cared for. The bleeding periods, method of bleeding, and analysis of blood were uniform. It was concluded that egg production has highly significant correlation with hemoglobin, calcium, inorganic phosphorus, carotene, and vitamin A. However, the egg production was average so normal changes in the blood should have been revealed. Fertility of eggs is not appreciably correlated with the blood picture studied and the fertility of eggs was also average. Hatchability of fertile eggs was below averages reported by other workers, but with average egg production and fertility of eggs it seems doubtful if the low hatchability was due to insufficient iron, calcium, phosphorus, carotene, or vitamin A. Therefore, the turkeys are considered to be average or normal.

Table 9 gives the mean, standard deviation, and range for the turkey hens; and the mean and range for the turkey

toms for the entire experimental period (December 28, 1943 to May 29, 1944) for hemoglobin, calcium, inorganic phosphorus, carotene, and vitamin A.

Table 9.--Normal blood picture for Broad-Breasted Bronze turkeys.

Blood Constituent	Turkey Hens			Turkey Toms	
	Mean	σ	Range	Mean	Range
Hemoglobin (gms./100 cc. blood)	10.90	± 0.99	7.1-14.7	13.33	12.3-14.4
Calcium (mgms/100 cc. plasma)	20.82	± 5.77	6.69-43.18	8.93	5.78-13.48
Phosphorus (mgms/100 cc. plasma)	6.06	± 1.43	2.48-9.75	4.01	2.55-4.58
Carotene (mgms/100 cc. plasma)	0.192	± 0.090	0.0277-0.683	0.352	0.125-0.606
Vitamin A (mgms/100 cc. plasma)	0.0760	± 0.022	0.0142-0.160	0.0771	0.0494-0.131

σ = standard deviation

In Table 9 a substantial difference between the mean hemoglobin, calcium, inorganic phosphorus, and carotene values between the turkey toms and the turkey hens is noted, however, the vitamin A values for the turkey toms and hens are in close agreement. The range is much greater for the turkey hens than for the turkey toms. In Figs. 1-4 this difference between blood levels for turkey hens and toms is apparent when the mean values for hemoglobin, calcium, inorganic phosphorus, and carotene are plotted against bleeding dates. Fig. 5 shows the close agreement of mean vitamin A values for turkey hens and toms on the various bleeding dates.

SUMMARY AND CONCLUSIONS

There were highly significant differences between dates for hemoglobin, calcium, inorganic phosphorus, carotene, and vitamin A and also between groups for hemoglobin, calcium, and vitamin A levels of blood from Broad-Breasted Bronze turkey hens. The highly significant differences between groups and dates accompanies variability in egg production between groups and dates.

The correlation coefficients were highly significant between egg production and hemoglobin, calcium, inorganic phosphorus, carotene, and vitamin A levels. However, there was no appreciable relation between egg production and fertility of the eggs.

It was concluded that the Broad-Breasted Bronze turkey hens used in this study were normal and the normal blood levels established for turkey hens prior to and including the laying period are:

Hemoglobin .....10.90 gm./100 cc. blood
Calcium .....20.82 mgm./100 cc. plasma
Inorganic Phosphorus..... 6.06 mgm./100 cc. plasma
Carotene ..... 0.192 mgm./100 cc. plasma
Vitamin A ..... 0.0760 mgm./100 cc. plasma

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The Author