EFFECT OF PHOSPHORUS SUPPLEMENTATION ON THE REPRODUCTIVE PERFORMANCE OF THE HEN AND THE CHEMICAL COMPOSITION OF THE EGG SHELL

by

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ABSTRACT

Eggs were obtained on consecutive days twice each week from each of five lots of S. C. White Leghorn Pullets (50 pullets/lot) that were fed a practical type basal diet supplemented with either soft phosphate with colloidal clay, a commercial graded dicalcium phosphate, or with a combination thereof. One lot receiving no supplemental phosphorus, served as a negative control. By analysis, the diets contained (%): protein 18.55 ± 0.49, calcium 2.30, phosphorus 0.722 ± 0.017 and 0.411 ± 0.033. Calculated inorganic phosphorus was 0.590 and 0.200 percent respectively.

The lot receiving no supplemental phosphorus had a significantly higher ($P < .01$) rate of egg production than the other lots tested. This was evidenced during the winter and summer months. The non-supplemented lot also had a better feed conversion when expressed on either a lb./doz. eggs or gm./gm. of egg basis. The values for this lot were 4.29 lbs., and 2.69 gm. respectively, while the averages for the other lots tested were 4.85 and 3.03 gm. respectively.

There was a greater loss of body weight among the birds in the lot receiving no supplemental phosphorus than in those fed the supplemented diets. This still did not affect the rate of egg production in the lot fed no supplemental phosphorus. There was no significant difference between treatments in the percent hatchability of fertile eggs. The lots

receiving the higher levels of dicalcium phosphate (Lots 7 and 9) had significantly heavier ($P< .01$) eggs than those in the other lots tested.

The differences observed in the internal quality of the egg could not be associated with dietary treatment, since when significant differences were observed they were without exception observed in the non supplemented lot and in at least one phosphorus supplemented lot.

Absolute and relative weights of shells from eggs in all treatments decreased, as previously reported, beginning with the fourth experimental month (April, 1958). This decline was apparent after the shell index, minimizing the effect of increasing egg size on percent shell, was computed.

The non-supplemented lot had a significantly higher percent shell, and shell index, when compared with the lot receiving all soft phosphate with colloidal clay.

Calcium, phosphorus, and protein determinations were carried out at monthly intervals on pooled samples of shells from the eggs used above. Calcium and protein content of the shells remained relatively constant, $36.85 \pm .06$ and $4.65 \pm .10$ percent respectively. Total phosphorus of shells declined sharply in all lots from 1127 ± 26 to 954 ± 30 ppm. Regression analysis indicated a significant difference in the rate of decline of shell phosphorus between the supplemented and non-supplemented lots. The rate of decline was curvilinear, being accelerated as the study progressed.

There was a correlation between the decline in percent shell and the decline in phosphorus. This correlation could not be reproduced physiologically when shells of different percentages were analyzed. No differences were observed between treatments in the percent bone ash or the calcium and phosphorus concentration of the shell.

INTRODUCTION: REVIEW OF LITERATURE

Need for Inorganic Supplement

Wheeler (1903), attempting to determine the value of minerals contained in commonly used feedstuffs of vegetable origin, added sand and ground Florida rock phosphate to day old chick diets. The growth of chicks fed the phosphorus supplemented diets was observed to be superior to that of birds fed the diet supplemented with only sand.

The results of Wheeler's work, indicating a possible need for mineral supplementation of poultry diets, received very little attention until Kennard (1919, 1921) demonstrated that a diet composed of grain and soybean oil meal will support satisfactory growth of chicks to eight weeks of age when supplemented with three parts of bone ash. Subsequent work by Phillips and Hauge (1925) indicated that soybean oil meal was a satisfactory source of protein for laying hens when the diet was supplemented with minerals.

The limited utilization of phosphorus from grains and other vegetable sources by some animal species was subsequently demonstrated by Bruce and Callous (1934), using rats. These workers recorded a low availability for phosphorus when it occurred in the form of inositol hexaphosphoric acid. The distribution of phosphorus in this form in plant materials was reported by Harris and Bunker (1935), who found that between 45 and 85 percent of the total phosphorus in 40 strains of corn, existed in the organically bound form. Later work by Heuser et al. (1943) suggested that a "reasonable quantity" of non phytin phosphorus must be included in the diet of chicks for normal growth and bone calcification.

Availability of Phytin Phosphorus

Phytic acid and its salts are collectively termed phytin. The phosphorus found in plants exists mainly in this form. In cereals and cereal by-products the phytin is found in the outer bran layers (Harris, 1955).

Lowe et al. (1939) reported that the addition of 0.369 percent phytin phosphorus to a basal diet, containing 0.41 percent inorganic phosphorus, did not improve calcification of the bones of chicks, whereas addition of equivalent amounts of dibasic sodium phosphate raised the bone ash ten percent. Singsen and Mitchell (1944) stated that plant protein concentrates may be used for growing stock without recourse to supplementary inorganic phosphorus, provided unheated leafy materials were incorporated into the ration, or access was provided to either green grass, leafy forage, or to range. They suggested that phytin phosphorus was efficiently utilized by chicks when the rations contained a sufficient amount of a phytin splitting enzyme phytase. This theory appeared to be untenable however, in view of the results of McGinnis et al. (1944) and Spitzer et al. (1945, 1948), who have shown that phytin utilization was not affected by the presence or absence of phytase in the diet, and that this enzyme was naturally present in the digestive tract of animals. Gillis et al. (1949) showed that replacement of an adequate level of available inorganic phosphorus by progessively higher levels of phytin, resulted in a rapid decline in growth and calcification, and in a marked increase in the Vitamin D requirements. However, the Vitamin D did not overcome the adverse effects associated with a low dietary level of non phytin phosphorus.

Phosphorus balance trials have been conducted with chicks during the age intervals of one to seven weeks, with diets containing 0.385 percent phosphorus. These diets were supplemented with various organic and inorganic sources of phosphorus (Nikolaiczuk, 1949). The mean phosphorus retention of calcium magnesium phytate was 16 percent, which was considerably lower than that of the orthophosphates tested. It has been generally assumed by most investigators that the phosphorus of the phytin molecule was very inactive in the body of the chick or the poult, and that the intact molecule passes out of the body in the feces (Common, 1939).

Singsen et al. (1950) showed that phosphorus of the phytin molecule, fed a phytin tagged with P^{32}, could move quickly and easily throughout the body, as was evidenced by the presence of P^{32} in the bone. However, this does not indicate that the phytin phosphorus has contributed to the net gain of the element for bone calcification since upon ashing of the bone low bone ash values were observed. It was concluded, therefore, that the phytin phosphorus merely enters into an exchange reaction with the existing phosphorus in the bone.

Gillis, et al. (1957) have isolated phytate as the calcium salt from kernels of corn which carried P^{32}. The corn was prepared by injecting a solution of P^{32} directly into the corn stalks as the grain approached maturity. Normal young chicks utilized P^{32} labeled calcium phytate only about one tenth as effectively as inorganic orthophosphate ($Na_2HP^{32}O_4$). In the presence of a Vitamin D deficiency the chicks did not make significant use of phytin phosphorus. However, the utilization of the inorganic source of the isotope by the chick was not impaired by Vitamin D deficiency.

The utilization of phytin phosphorus by laying hens has not been adequately defined. Common (1939) obtained evidence that a large part of phytic acid in the diet of laying hens remained unhydrolyzed as it passed through the digestive tract. To give support to Common's work, Gillis et al. (1953) showed that phytin phosphorus was not as satisfactory as inorganic phosphorus in meeting the requirements of laying birds. This was true when purified calcium phytate and phytin found in common feedstuffs of plant origin were used. It was estimated that phytin phosphorus was utilized about one half as well as dicalcium phosphate. O'Rourke *et al.* (1954) constructed a semipurified diet containing 0.19 percent phosphorus, of which 0.12 percent was phytin phosphorus. This ration did not support "normal" egg production and hatchability. O'Rourke *et al.* (1955) showed that normal egg production and hatchability could be maintained on a basal diet containing 0.43 percent total phosphorus; however, the percent of phytin phosphorus present in the diet was not given.

Pepper *et al.* (1958) showed that hens maintained "normal" egg production and egg shell quality when fed a basal diet containing no supplemental phosphorus; however, again the percent of phytin phosphorus present was not given.

Availability of Supplemental Inorganic Phosphorus

Since early investigators have shown that phosphorus in the organic form, as found in common feedstuffs, was not utilized to any great extent by the chick, attention has been directed to inorganic supplementation. Much work has been done to determine the best inorganic supplement. Initial work by Bird and Caskey (1943), using a low phosphorus practical type basal diet and bone ash as a measure of phosphorus availability, indicated that amorphous calcium metaphosphate was a satisfactory supplement when compared with tricalcium phosphate. Gerry *et al.* (1946) showed that raw rock phosphate and colloidal phosphate retarded growth of chickens under eight weeks of age; however, this was overcome at an early age, when the diet contained less than 0.068 percent fluorine. Superphosphates and rock phosphates were found to be high in fluorine. These phosphorus supplements were detrimental to growth if they contained more than 0.38 percent of fluorine (Gerry et al., 1947).

Using growth and percent bone ash as criteria for measuring availability of phosphorus in chicks, Singsen and Scott (1946) revealed that sodium acid phosphate, tricalcium phosphate, and bone meal, were of equal value, and readily available to the chick. Gillis et al. (1948) reported that orthophosphates, including potassium acid phosphate, pure beta and tricalcium phosphate, and reagent grades of mono-, di-, and tricalcium phosphates were excellent sources of phosphorus, and were slightly more available than steamed bone meal. The defluorinated superphosphate and defluorinated phosphate rock products were found to be good sources of phosphorus, but slightly less available than the pure orthophosphates or steamed bone meal. Further evidence of the availability of bone meal, dicalcium phosphate, defluorinated phosphate, and Curacao Island phosphate, was furnished by Miller and Joukovsky (1953). Grau and Zweigart (1953) found that phosphatic clay is not as valuable a source of phosphorus as is bone meal. These workers concluded that the growth limiting factor was not associated with fluorine, because when sodium fluoride was added to a diet supplemented with tricalcium phosphate, it did not depress growth or affect calcification.

Using toe ash determinations for measuring the degree of calcification, Johnson et al. (1953) found that feeding four percent soft phosphate (0.36% phosphorus) with colloidal clay produced satisfactory calcification and growth. A similar growth response occurred with a two percent level (0.24% phosphorus) of steamed bone meal. Using a practical type diet, Gillis et al. (1951) concluded that the phosphorus in colloidal phosphate was about one fourth as effective as orthophosphates, for chick growth and bone calcification.

A solubility procedure for measuring the availability of various phosphorus supplements was developed by Reynold et al. (1944). The availability was based on their solubility in 0.4 percent hydrochloric acid. The solubility of hydrated dicaclium phosphate was found to be 100 percent, that of alpha tricalcium phosphate 77.6 percent, and beta tricalcium 76.8 percent. The solubility of two steamed bone meal samples was 98.2 and 100 percent, whereas that of defluorinated phosphates ranged from 29.2 to 94.2 percent. Colloidal phosphate was found to be 69.2 percent soluble.

A biological assay procedure was developed by Gillis et al. (1951), who used a blood fibrin-cerelose purified type diet, containing about 0.05 percent total phosphorus, to compare the relative availability of a number of common phosphorus supplements. The growth and bone ash of chicks fed several phosphorus supplements were compared to those of chicks fed tricalcium phosphate which had been assigned the biological value of 100. All orthophosphates were found to be readily available. Bone meals were good sources of phosphorus. Most of the pyrophosphates were completely unavailable, as were the metaphosphates. The authors pointed out that the HCl method of estimating availability had only limited usefulness. Solubility could not be taken as an indication of biological availability. It was pointed out that the rather soluble alpha, beta, and gamma calcium pyrophosphates were completely unavailable biologically.

Motzok et al. (1956) studied the availability of twenty-seven phosphorus supplements of both reagent and commercial grades. These workers pointed out that soft phosphate with colloidal clay was not a standardized product, and that bone ash and growth response were not always of the same sensitivity when measuring the biological availability of phosphorus. This was evidenced when five samples of soft phosphate with colloidal clay were obtained from different producers. Four samples gave growth and bone ash values significantly below those observed with tricalcium phosphate supplemented diets. One sample gave a growth response significantly below tricalcium phosphate, but the percent bone ash was not significantly different. As judged by percent bone ash and body weight, it was found that tricalcium phosphate, monocalcium phosphate, dicalcium phosphate, monosodium phosphate, disodium phosphate, Curacao Island phosphate, sodium tripolyphosphate, and acid sodium pyrophosphate, were readily available.

The availability of phosphorus to laying hens has not been studied as extensively as with chicks. Halpin and Lamb (1932) reported that high levels of raw rock phosphate and phosphatic limestone had a depressing effect upon egg production. Evans and Carver (1943) showed that defluorinated phosphate gave as "satisfactory" egg production as bone meal, when the same percentage was included in the diet. The availability of soft phosphate added to a basal practical type diet was compared with a diet not containing supplemental phosphorus, and also to a diet containing dicalcium phosphate, by Pepper et al. (1958). These workers showed that there was no difference in egg production, feed required per dozen eggs, body maintenance, or egg shell quality.

Phosphorus Requirement of the Chick and Laying Hen

Wilgus (1931) reported that the requirement for total phosphorus by the chick was 0.5 percent of the diet or less. The author also observed that the calcium--phosphorus ratio could vary between 1.0:1 and 2.2:1, with normal results, but if the ratio was 3.3:1, the results were disastrous. The phosphorus requirement was higher during the first six to eight weeks of growth than for the ensuing twelve weeks when phosphoric acid was used as the phosphorus supplement (Sherwood, 1932). The calcium--phosphorus ratio in the bone was found, by Haag (1939), to progressively increase from 1.25 in day old chicks, to 1.73 in birds 20 weeks old. The National Research Council (1937) listed the minimum requirement for calcium at 0.7 percent, but stated that about twice this amount was desirable. The minimum total phosphorus requirement for all ages was found to be about 0.4 percent or 0.5 percent, allowing for a margin of safety. It was reported by Singsen (1946) that 0.45 percent non phytin phosphorus in the diet would support excellent growth and bone calcification in the chick. Optimal results were obtained by Carver et al. (1946) when the diet contained 1.6 percent calcium and 0.8 percent total phosphorus. Gillis et al. (1949), using purified and practical type diets, concluded that optimum early growth and bone formation were obtained with 0.6 percent total phosphorus in the diet, of which 0.4 percent had to be non phytin phosphorus. Using a natural diet with some phytin phosphorus, O'Rourke et al. (1952, 1955) demonstrated that the requirement for total phosphorus decreased with age. At least 0.73 percent was needed for chicks up to three weeks of age; 0.6 percent from four to ten weeks, and not more than 0.42 percent from ten weeks to sexual maturity.

The phosphorus requirement of laying pullets has not been established. Branion (1938) concluded that mineral supplements should be fed ad libitum due to the disparity in the calcium and phosphorus requirements as reported by various investigators. Approximately 0.80 percent total phosphorus was required for optimum production when the calcium content of the diet was fixed at either 2.23 or 3.00 percent (Miller and Bearse, 1934).

The work of Norris et al. (1934) indicated that 0.5 percent total phosphorus was not sufficient for egg production, but 0.75 percent was adequate. Mitchell and McClure (1937) expressed the requirement by standardizing the egg production at 75 percent, and phosphorus availability at 0.5 percent. Under those conditions the requirement was calculated to be 0.35 percent total phosphorus. Schaible (1941) reviewed the literature and concluded that 0.4 percent total phosphorus was required, but to allow for a margin of safety, 0.5 percent was recommended.

Using egg shell thickness and production to determine the most satisfactory levels of calcium and phosphorus for laying hens, Evans and Carver (1942) stated the total phosphorus requirement in terms of the amount of calcium present in the diet. When 1.5 percent calcium was present, 0.6

percent total phosphorus was adequate, but if 2.5 percent calcium was included in the ration, 0.8 percent total phosphorus was required. When 3.00 percent calcium was included, 0.8 percent phosphorus was not as satisfactory as 1.0 percent. Evans et al. (1944) concluded that 0.6 percent of total phosphorus was not adequate.

Since a practical type diet was employed by the above investigators, the phosphorus content of the ration was not sufficiently low. O'Rourke et al. (1954), therefore, formulated a semipurified diet containing 0.19 percent total phosphorus. This diet did not support "normal" egg production or hatchability. When the basal ration was supplemented with dibasic calcium phosphate, to contain 0.3 percent total phosphorus, "normal" production and hatchability were maintained. This diet contained 0.18 percent non phytin, and 0.12 percent phytin phosphorus. O'Rourke et al. (1955) concluded that not more than 0.42 percent total phosphorus was needed for laying pullets on a practical type diet. Using egg production and maintenance of body weight as criteria for phosphorus requirements, Gillis et al. (1953) stated that it appeared that the hen needed 0.5 percent available phosphorus. For maintenance of optimum blood phosphorus levels and for the prevention of decalcification of bone, the requirement was approximately 0.6 percent available phosphorus. Pepper et al. (1958) found that hens fed a diet containing 0.43 percent total phosphorus performed as well as those whose diets were further supplemented with either dicalcium phosphate or with soft phosphate with colloidal clay.

The Role of Phosphorus in Shell Formation

It has been known for some time that the quality of an egg shell is influenced, among other factors, by : (1) heredity, (2) seasonal variations, and (3) nutrition. Taylor and Lerner (1939) were able to establish thick and thin egg shell laying strains. Taylor and Martin (1928), and Hays (1937), concluded that hereditary influences probably accounted for the inability of some hens to produce thick shelled eggs even under the most favorable conditions. Wilhelm (1940) showed that the decline in thickness of the shell was correlated with the rise in prevailing temperatures. Thinner egg shells were obtained "immediately" after experimentally increasing the environmental temperature from 20 C to 32.5°C (Warren and Schnepel, 1940). A recovery in thickness was reported after subsequent decrease in temperature. Conrad (1939) postulated that the occurrence of soft shells during high temperatures might be due to either a decreased capacity of the blood stream to carry calcium, or to a decreased intake of dietary calcium.

The mineral elements shown to affect shell quality are calcium (Evans *et al.*, 1944a; Norris *et al.*, 1934; and Evans *et al.*, 1944b), and manganese in small amounts (Lyons, 1939).

Very little information has been made available concerning the role of phosphorus in shell quality.

Taylor and More (1956) showed that seven to ten days before a pullet begins to lay, a new system of secondary bone was laid down under the combined influence of androgens and estrogens, in the marrow cavities of all the bones in the skeleton in which haematopoietic tissue was present. This medullary bone forms a highly labile reserve of minerals which are mobilized during the calcification of the egg shell. Using Ca^{45} as a tracer, Jowsey et al. (1956) showed that a laying hen depositing calcium on the shell will obtain about 35 percent of the calcium for this shell from the food in the gut. Evans and Carver (1942) found that 2.5 percent or higher of calcium prevented a decrease in egg shell thickness only if the level of phosphorus was satisfactory.

There still seems to be some question as to the amount of phosphorus found in the shell. Stewart (1935) stated that it was well established that there was 0.73 percent P_2O_5 (0.32 phosphorus) in the egg shell. Romonoff and Romonoff (1949) listed the egg shell as containing 0.9 percent elemental phosphorus. Almquist and Burmester (1934) gave the phosphorus content of the shell at around 0.15 percent PO_4 (0.054% phosphorus). Smith et al. (1954) found that there was approximately 0.125 percent phosphorus in the shell as elemental phosphorus.

Almquist and Burmester (1934), in describing a "glassy" egg shell which would normally fall into the commercial thin shell grade, stated that this abnormal shell was slightly lower in $CaCO_3$. Otherwise there was no difference in the mineral composition of this shell and a normal shell. However, when the phosphorus values of glassy shells were averaged and compared with those of the normal shells, the percent phosphorus was 0.100 and 0.127 percent, respectively.

Common (1936) showed that a heavy excretion of phosphorus was associated with egg laying. This did not necessarily mean a mobilization of bone minerals. It was probably due to the immediate demand for dietary calcium in the formation of the egg shell, because he also showed that when sufficient calcium carbonate was fed there was no increase in serum phosphorus. It might also be pointed out that, although Common makes no mention of it, when a thin shelled egg was induced experimentally by withholding the calcium, the absolute value of phosphorus in grams decreased, and also the relative value of phosphorus per gram of shell decreased. This is in direct contrast to the work of Buckner _et al._ (1923), who pointed out that as the egg shell became thinner, the percentage composition remained constant. Lorenz _et al._ (1938) found that upon injecting hens with P^{32} it took one hour to get the maximum activity in the shell. Shells from eggs layed one hour after injection were found to contain 0.15 percent of the injected dose. The shell contained 0.59 percent of the injected dose after six hours. Smith <u>et al.</u> (1954) showed that less than two percent of the injected dose of P^{32} was transferred to the shell the first twelve days, except when laid soon after injection. One shell laid two hours after injection contained two percent of the injected dose. It was pointed out that this indicated that a large part of the shell phosphorus was deposited even after mineral deposition was complete.

In view of the limited information on (a) the utilization of phosphorus from soft phosphate with colloidal clay by the hen, and (b) the biochemical changes in shell composition associated with shell quality, the studies reported herein have been initiated.

EXPERIMENTAL PROCEDURE

Prior to sexual maturity (20 weeks), 250 S. C. White Leghorn pullets of similar breeding, randomly chosen from the University flock, were distributed among five floor pens of 50 pullets each. Each pullet was individually identified by means of leg bands and plastic type wing badges.[1]

Each pen was equipped with an automatic water supply, metal feed troughs, roosts, and trap nests. Straw was used as a source of floor litter. A minimum of fourteen hours of total light (morning light) per day was provided throughout the duration of the study. Maximum and minimum temperatures were recorded daily.

Pullets were placed on the basal diet (Table 1), supplemented with the phosphorus sources shown in Table 2, on December 5, 1957, and remained on these diet until termination of the experiment, August 15, 1958.

Dynafos (commercial dicalcium phosphate) and soft phosphate with colloidal clay were chosen for this study because of their wide difference of availability of phosphorus for chick growth, as reported in the literature (Gillis _et al._, 1948; Motzok _et al._, 1956). Analysis of Dynafos shows that it contains approximately 22.44 percent

1 Dryden Poultry Breeding Farm, P. O. Box 951, Modesto, California.

Table 1

COMPOSITION OF BASAL DIET

Ingredients	%
Ground Yellow Corn	20.00
Ground Milo	35.16
Ground Wheat	10.00
Alfalfa Meal	5.00
Soybean Oil Meal (Solvent extracted)	17.70
Fish Meal	2.50
Whey	1.25
NaCl	0.20
$CaCO_3$ [1]	5.65
$MnSO_4$ [2]	0.04
Soft Phosphate with Colloidal Clay[3]	-----
Dicalcium Phosphate[3]	-----
Vitamin Premix[4]	2.50

1 Ground limestone.

2 Tecmangam (70% $MnSO_4$), Eastman Kodak Company, Rochester 3, N. Y.

3 Phosphorus supplement added at the expense of ground milo.

4 Supplied the following per kg. of mixed diet; 9,900 I.U. Vitamin A, 1,540 I.C.U. Vitamin D_3, 4.40 mg. Riboflavin, 27.50 mg. Niacin, 11.00 mg. D-Calcium Pantothenate, 440 mg. Choline Chloride, 013 mg. Vitamin B_{12}, 5.50 mg. Vitamin E, 2.20 mg. Vitamin K, 4.40 mg. Procaine Penicillin, 22.20 mg. Aureomycin, 124.85 mg. BHT, 998.8 mg. DL-Methionine.

Table 2

LEVELS OF SUPPLEMENTAL PHOSPHORUS IN EXPERIMENTAL DIETS

Lot No.	No. of Hens Started	No. of Males	Supplements to Basal Diet		Phosphorus Concentration[3]	
			Soft Phosphate[1] With Colloidal Clay (gm./kg.)	Dicalcium[2] Phosphate (gm./kg.)	Total (%)	Available[4] (%)
6	50	4	45.00	-----	0.820	0.590
7	50	4	27.50	8.30	0.860	0.590
8	50	4	13.75	14.80	0.820	0.590
9	50	4	-----	21.30	0.803	0.590
18	50	4	-----	-----	0.420	0.200

1 Kelfos, Soft Phosphate Research Institute, Inc., Ocola, Florida.

2 Dynafos, International Minerals and Chemical Corp., Skokie, Illinois.

3 Calculated according to Titus, H. W., (1955).

4 Animal and supplemental phosphorus was considered to be inorganic and 100 percent available. Approximately 30% of the phosphorus in plant products was considered to be non phytin, and was included in the total inorganic phosphorus (National Research Council, 1950).

calcium, 18.84 percent phosphorus, 1.88 percent iron, 1.14 percent aluminum, 0.12 percent fluorine, 2.90 percent hydrogen, and 52.08 percent oxygen (Harwood, 1956).

In the phosphate industry soft phosphate with colloidal clay is generally known as waste pond phosphate inasmuch as it is the waste product from washing or desliming phosphate rock. It contains finely divided phosphate together with large amounts of clay and other impurities (Sanchelli, 1951). Motzok et al. (1956) stated that soft phosphate with colloidal clay contained 7.6 to 11.3 percent phosphorus. However, in the study reported herein the soft phosphate contained 9 percent phosphorus. The phosphorus, as determined by paper chromatography, existed as ortho and pyrophosphate in about equal quantities, and possibly a third phosphate (a tri phosphate). They also stated that the impurities in soft phosphate consisted of approximately 19.5 percent complex silicates and undetermined matter, 3.9 percent aluminum, 3.3 percent iron, and 1.4 to 1.7 percent fluorine.

Diets were mixed at two week intervals in 300 pound quantities and stored in cans. Soybean oil meal (solvent extracted) was used as a diluent in premixing the vitamins. A monthly record was kept on the feed consumed by each lot. Twice during the course of the experiment the diets were sampled for calcium (A.O.A.C., 1950), phosphorus (Koenig and Johnson, 1942), and nitrogen (Kirk, 1950) analyses (Table 3).

Table 3

COMPOSITION OF EXPERIMENTAL DIETS

	Lot 6	Lot 7	Lot 8	Lot 9	Lot 18
Crude Protein (%)[1]					
Calculated	17.06	17.00	17.00	17.00	17.00
Analyzed	17.92±.24	18.07±.25	18.92±.60	19.21±.26	18.63±.36
Crude Fat (%)	2.31	2.34	2.33	2.39	2.40
Crude Fiber (%)	4.28	4.27	4.15	4.28	4.29
Total Calcium (%)					
Calculated	2.52	2.54	2.49	2.52	2.48
Analyzed	2.31±.08	2.30±.12	2.30±.16	2.31±.14	2.30±.11
Total Phosphorus (%)					
Calculated	0.820	0.820	0.820	0.803	0.420
Analyzed	0.709±.021	0.716±.028	0.712±.028	0.750±.024	0.411±.033
Productive Energy Cal./kg.	1931.6	1956.9	1947.9	1982.4	1990.6
C/P	51.5	52.3	52.1	53.0	53.2

1 N X 6.25

Feed and water were supplied *ad libitum*.

The birds were individually weighed to the nearest gram at the beginning of the study, and at the close of each experimental period thereafter. An accurate record of mortality was kept. Dead birds were taken to the University Pathology Laboratory for diagnosis. Eggs produced during the first three days of each experimental month were set in a Jamesway incubator, and a record of the percent hatchability and fertility was kept.

Eggs were collected twice daily, and were weighed in baskets. Percent production was calculated each month, and expressed on a "hen day" and "hen housed" basis. In addition, hens were individually trap-nested the first two days of each week. Eggs so collected were weighed individually to the nearest tenth of a gram on a Mettler K-5 automatic balance.[2] These values were used to calculate the average egg weight, and to determine the distribution of eggs into the weight classes as defined by the U.S.D.A. (Federal Register, 1955).

After weighing, eggs were broken out on a glass stand and the quality of the albumin and yolks were noted. Albumin height was determined according to the method of Kilpatrick *et al.* (1958) using an Ames S-5677 Micrometer.[3] Degree of yolk mottling was scored using a 0 to 10 scale as outlined

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2 Braun Corporation, P. O. Box 1431, Phoenix, Arizona.

3 B. C. Ames Company, Waltham 54, Massachussetts.

by Baker _et al._ (1957). The presence of blood spots was noted and further classified as "large" or "small", small denoting spots that would normally not be detected upon candling (usually below 1/8 inch in diameter). The weight of the egg and the albumin height were used to calculate the Haugh Index, by means of an Interior Quality Calculator[4] (Haugh, 1937). In the event that the thick albumin was broken, its measurement was not included in the total calculations. Distribution of eggs into market quality classes was done according to U.S.D.A. recommendations (Federal Register, 1955).

The shells, (shell plus membrane and cuticle, unless otherwise stated) were subsequently washed free of albumin, air dried, and weighed to the nearest hundredth of a gram. Dried shells collected for each lot were ground at monthly intervals and a representative sample retained for analysis. Three to four samples were taken from each lot, and analyses carried out in triplicate. Nitrogen was determined by the method of Kirk (1950), and calcium and phosphorus, by the methods of A.O.A.C. (1950), and Koenig and Johnson (1942), respectively. Aliquots used for Ca and P determinations were digested with a mixture of concentrated nitric, perchloric (72%), and water, as outlined by Piper (1950).

During the course of the experiment it was found desirable to separate the shell membrane from the shell, in

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4 U.S.D.A. Agricultural Marketing Service, Washington, D. C.

order to determine the relative distribution of the constituents between these parts of the egg. Due to labor and time involved in separating the membranes from the "inorganic portion of the shell", this was only done once during the course of the study, as noted below.

Eggs were collected for three consecutive days during the ninth experimental month, individually broken, and the shells treated as outlined previously. The membrane was removed from half of each shell by pulling it away from the shell with the aid of forceps. The calcium, phosphorus, and nitrogen concentrations were determined on the membrane, membrane free shells, and the shell plus the membrane, using the methods previously outlined. Due to the difficulty of ascertaining whether the shell was completely freed from its membrane, the analytical results were expressed in relative terms, i.e., percent.

In view of the results obtained by the chemical analysis of pooled samples of egg shells, it was found desirable to analyze individual shells for their calcium, phosphorus, and nitrogen content. Eggs were collected during the ninth experimental month, and individually prepared for analysis as previously described.

Upon termination of the experiment, ten hens chosen at random from each lot were sacrificed, and the left tibias removed for ash determination, according to A.O.A.C. (1950), using Skellysolve B as the solvent. The ash of at least three bones from each lot were analyzed for calcium and phosphorus by methods outlined above.

The data was analyzed by an analysis of variance, and the differences tested by the Duncan Multiple Range Test (Biometrics 11:1-42, 1955).

CHAPTER I RESULTS AND DISCUSSION: THE EFFECTS OF PHOSPHORUS SUPPLEMENTS ON THE RATE OF EGG PRODUCTION AND THE INTERNAL QUALITY OF EGGS OF S. C. WHITE LEGHORN PULLETS

Egg Production

Total average egg production for each lot for the entire experimental period is summarized in Table 4. With the exception of Lot 8, the relative levels of egg production as calculated on a "hen housed" (H.H.D.) basis were similar to those expressed on a "hen day" (H.D.) basis. The lower values associated with the former are due to the level of mortality which influences its calculation. Hen housed days were calculated on the basis of the number of hens originally started, times the number of days in the experimental period, whereas hen days were calculated on the basis of the number of remaining birds after mortality was considered, times the number of days in an experimental period (Table 4). Since the mortality could not be attributed directly to the phosphorus treatments (Table 9), the values calculated on a "hen day" basis were used for statistical analysis (Table 4a).

When expressed on a "hen day" basis, no significant differences ($P > .05$) were observed among the phosphorus supplemented lots. However, hens in Lot 18, receiving no supplemental phosphorus, showed a significant increase ($P < .01$) in egg production over those fed the phosphorus

Table 4

EFFECT OF PHOSPHORUS SUPPLEMENTS ON THE EGG PRODUCTION AND FEED CONVERSION OF S. C. WHITE LEGHORN PULLETS

Lot No.	Supplements to Basal Diet (gm./kg.)		Average Total Egg Production			
					Feed Conversion	
	Soft Phos.	Dical. Phos.	H.H.D.[1]	H.D.[2]	lbs/doz Eggs	gm/gm Eggs
6	54.00	-----	54.49	59.19	4.98	3.15
7	13.75	14.80	57.25	62.36	4.79	2.98
8	27.50	8.30	56.86	63.43	4.74	3.00
9	-----	21.30	56.14	60.74	4.88	2.99
18	-----	-----	63.20	69.06**	4.29	2.69

1 Hen Housed Days=(No. of birds started) x (No. of days in experimental period).

2 Hen Days=(No. of remaining birds) x (No. of days in experimental period).

** $(P < .01)$

Table 4a

ANALYSIS OF VARIANCE OF THE EFFECTS OF PHOSPHORUS SUPPLEMENTS ON EGG PRODUCTION

Source of Variation	d f	Mean Square
Season	1	1,387.78
Period in Season	7	153.95
Treatments	4	129.02**
Treatments x Season	4	24.99
Treatments x Period in Season	28	11.80

** ($P < 0.01$)

supplemented diets (Lots 6, 7, 8 and 9). The phosphorus supplemented lots had a total phosphorus content of 0.722 ±0.017 percent whereas the non-supplemented lot had a total phosphorus content of 0.411 ±0.033 percent (Table 3).

The requirement for total phosphorus by the hen in the presence of approximately 2.3 percent calcium, was shown to be 0.6 to 0.8 percent of the total diet (Miller and Bearse, 1934; Norris et al., 1934; Evans and Carver, 1942; and Gillis et al., 1953). These workers used a practical type diet composed of ground grains and a vegetable and an animal source of protein, supplemented with the recommended levels of vitamins and minerals. When a diet of similar composition (Table 1) as that fed by the workers cited previously, consisting of 0.722 ±0.017 percent total phosphorus and 2.30 percent calcium (Table 3), was fed to hens in this study, a marked depression in egg production was observed (Lots 6, 7, 8 and 9, Table 4).

Results reported here coincide with those of later workers (Pepper et al., 1958, and O'Rourke et al., 1955), who were unable to improve egg production by supplementing diets of laying pullets, already containing either 0.38 or 0.43 percent total phosphorus, with inorganic sources of this element. Pepper et al. (1958) observed no change in the rate of egg production when hens were fed a basal diet containing 0.38 percent total phosphorus that was supplemented with either dicalcium or soft phosphate to supply 0.05 to

0.1 percent additional phosphorus. This could be explained by the fact that the phosphorus content of the diet used by the above workers did not exceed 0.48 percent. However, O'Rourke et al. (1955) noted a decrease in egg production when an "all vegetable protein" basal diet containing 0.43 percent total phosphorus was supplemented with inorganic phosphorus to contain more than 0.6 percent total phosphorus. The two previous experiments utilized "all vegetable protein" diets, whereas the diets used in this study (Table 1) contained 2.5 percent fish meal, which was a readily available source of inorganic phosphorus.

Additional explanation for the better performance of the laying hens on the lower than recommended level of phosphorus in this study, and also in the studies of previous workers, was offered by Gillis et al. (1953), who indicated that the laying hen possibly had greater ability to utilize phytin phosphorus than the young chick. This may explain the existing discrepancies regarding the phosphorus requirements of the adult chicken.

In view of the high level of egg production observed among the hens in the non-supplemented group (Lot 18), it is evident that the basal diet used in this study containing 0.41 percent total phosphorus, was not suitable for measuring the relative availability of the various phosphorus supplements for egg production.

Egg production for winter and summer periods are summarized in Table 5. Analysis of variance (Table 4a) indicated that the season had a significant effect ($P < .01$) on the rate of egg production in all lots. Treatment times season interaction, however, was found not to be significant ($P \geq .05$), indicating that the differences observed among treatment groups were consistent from season to season.

The lot supplemented with only soft phosphate (45 gm./kg., Lot 6) had a significantly lower ($P \leq .01$) rate of egg production during the winter period than did any of the other lots tested. However, the lot receiving no supplemental phosphorus (Lot 18) had a significantly higher ($P < .01$) rate of egg production than did any of the supplemented lots.

Average egg production declined sharply in all lots during the summer months. The adverse effect of high temperatures on the rate of egg production has been shown by Wilhelm (1940), and Conrad (1939). With the exception of Lot 6, the relative decline was greater among the supplemented lots (16.7 to 22.2%), than in the negative control (12.7%). Lot 6 showed the least relative decline (9.33%) between seasons. This was due to the low rate of production in this lot during the winter months. Although during the winter months Lot 6 had a significantly lower ($P \leq .01$) rate of egg production than any of the other lots tested, there was no significant difference between this lot and the other supplemented lots during the summer months. The lot receiving

Table 5

EFFECT OF PHOSPHORUS SUPPLEMENTS ON THE EGG PRODUCTION OF S. C. WHITE LEGHORN PULLETS DURING THE WINTER AND SUMMER PERIODS

Lot No.	Supplements to Basal Diet (gm./kg.)		Average Egg Production	
	Soft Phos.	Dical. Phos.	Winter[1] (%)	Summer[2] (%)
6	45.00	-----	62.50a[3]	55.88a[3]
7	13.75	14.80	68.09b	56.63a
8	27.50	8.30	69.09b	55.77a
9	-----	21.30	68.33b	53.15a
18	-----	-----	73.73c	64.40b

1 December through March

2 April through August 15

3 Means with different subscripts are significantly different.

no supplemental phosphorus (Lot 18) had a significantly higher ($P < .01$) rate of egg production during the summer months than any of the supplemented lots.

The changes in the monthly rate of egg production in relation to mean maximum and minimum laying house temperatures are shown in Figure 1. Since maximum environmental temperatures began to rise sharply during the month of April, this month was chosen as the beginning of the "summer" season (Table 6).

The difference between mean monthly maximum and minimum temperatures varied from 18.2 to 31.7°F during the course of study, while the average variation between winter and summer season was 26.5 and 24.9°F, respectively. The summer season, however, showed the greatest difference between mean monthly maximum and minimum temperatures within the season. These differences ranged from 18.2 to 31.7°F for the summer season, while the differences ranged from 23 to 31.7°F for the winter season. The highest average maximum temperatures during the course of study were reached in the month of June (102.2°F).

Egg production of all the supplemented lots declined during the first three months of the study (December through February), and then remained relatively constant until the month of June, whereas for the same period, egg production for the lot receiving no supplemental phosphorus (Lot 18) remained relatively constant (Figure 1). During the month

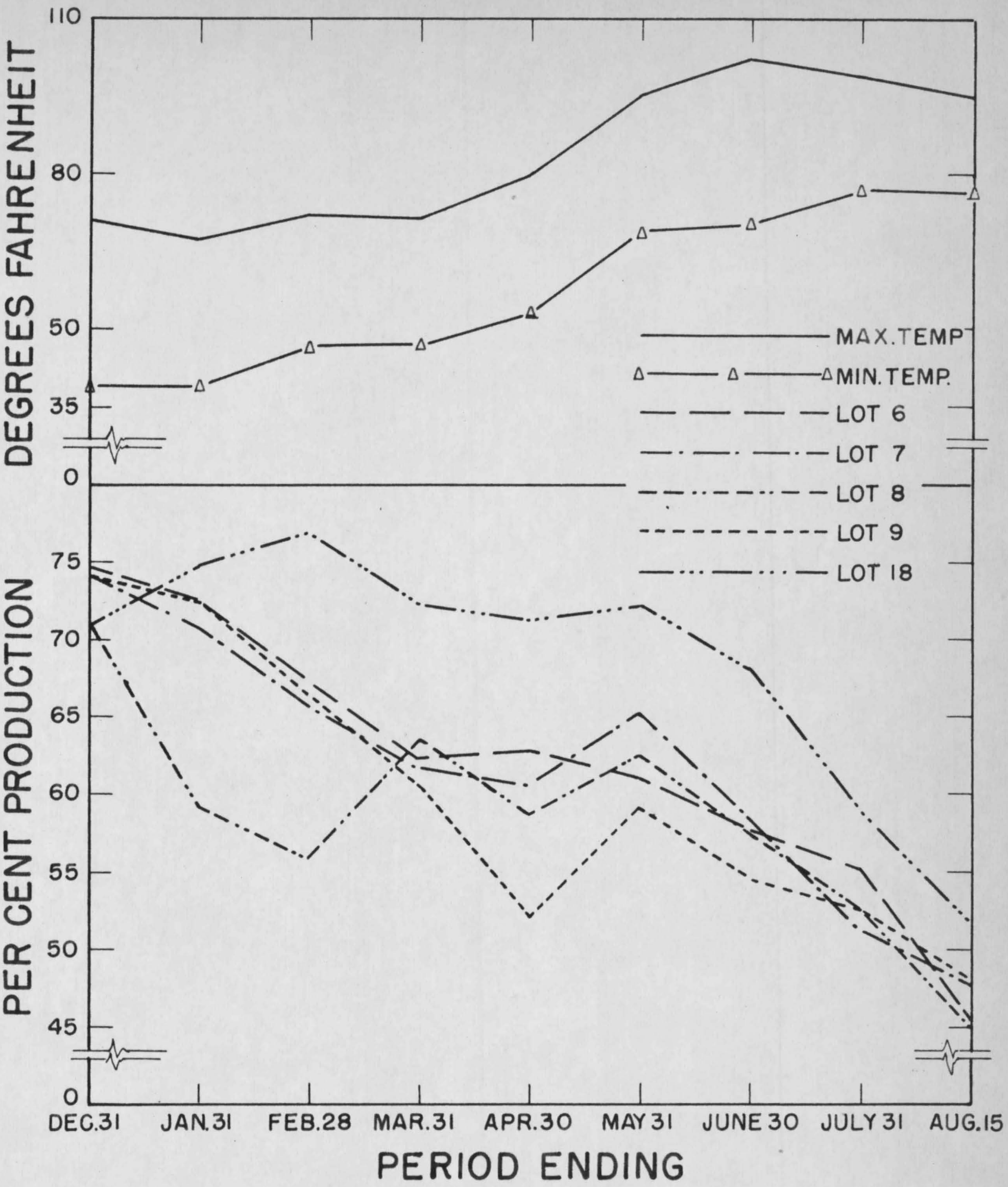

Fig. 1—The average maximum and minimum laying house temperatures during the experimental period and the effect of temperature upon average rate of egg production.

Table 6

SUMMARY OF AVERAGE MONTHLY TEMPERATURES

Period Ending	Maximum[1]	Minimum[2]
12/31/57	70.0	39.0
1/31/58	66.3	39.1
2/28/58	71.5	46.5
3/31/58	70.4	47.4
4/30/58	79.9	52.5
5/31/58	95.3	69.1
6/30/58	102.2	70.5
7/31/58	98.2	77.1
8/15/58	94.4	76.2

1 Recorded after 4:00 p.m.

2 Recorded before 8:00 a.m.

of June, egg production declined sharply in all lots and continued to decrease until the termination of the study.

Feed Conversion

Feed conversion when expressed on a "lb./doz. eggs" basis, paralleled the level of egg production (Table 4). Feed conversion is usually reported in these terms (MacIntyre and Jenkens, 1955, and Heywang, 1956). It was noted that if small differences are considered, feed conversion when expressed on a "gm./gm. eggs" basis, is not affected by the rate of egg production as strikingly as when it is expressed in the form of "lb./doz. eggs". However, the lot receiving no supplemental phosphorus (Lot 18) had better feed conversion when expressed on either a "lb./doz. eggs" or "gm./gm. eggs" basis.

Body Maintenance

Changes in body weights are summarized in Table 7. All lots showed a decline in body weight when compared with the average initial weight of the bird. Decline in body weight was evident during both the winter and summer periods. Lots supplemented with the higher levels of dicalcium phosphate were able to maintain a more stationary weight (Lot 7 and 9), than the lots receiving higher levels of soft phosphate (Lot 6 and 8). The lot receiving no supplemental phosphorus (Lot 18) had the greatest weight loss.

Table 7

EFFECT OF PHOSPHORUS SUPPLEMENTS ON AVERAGE BODY WEIGHTS

Lot No.	Supplements to Diet (gm./kg.)		Initial Weight (gm.)	Winter Weight (gm.)	Summer Weight (gm.)	Weight[1] Loss (gm.)
	Soft Phos.	Dical Phos.				
6	45.00	-----	2145	2053	1946	180
7	13.75	14.80	2137	2104	2074	75
8	27.50	8.30	2067	2034	1954	170
9	-----	21.30	2089	2070	2011	46
18	-----	-----	2120	2071	1949	264

1 Initial weight minus final weight

Gillis et al. (1958) reported that when the diets of laying hens contained less than 0.5 percent available phosphorus, body weight and egg production declined sharply. The authors concluded that at least 0.5 percent available phosphorus was required for body weight maintenance. In the study reported here, the hens receiving no supplemental phosphorus showed the greatest loss in weight (Lot 18, Table 7). Nevertheless, under the conditions of this study, maintaining body weight apparently was not necessary for maximum egg production, since Lot 18, which received no supplemental phosphorus, had the highest level of egg production, and also the greatest loss in weight.

Fertility and Hatchability

Percent fertility and hatchability are summarized in Table 8. Total hatchability has been known to be influenced by a number of factors, including the fertility of the eggs. In Lot 18 (Table 8), the fertility remained low until the fifth experimental month. This was probably due to the death of two males during the month of February because, following the replacement of the males (May 13, 1958) the fertility in this lot increased to that of the other lots. In view of the fact that some of the males in Lots 7, 8, 9, and 18, died, the percent hatchability of fertile eggs, rather than that of total eggs set, was considered for statistical analysis. No significant differences ($P > .05$) were observed

Table 8

EFFECT OF PHOSPHORUS SUPPLEMENTS ON FERTILITY[1], HATCHABILITY, AND PHOSPHORUS CONTENT OF THE YOLK OF S. C. WHITE LEGHORN PULLETS

Lot No.	Number of Eggs Set			Fertility[6]			Hatchability of Fertile Eggs			Total Hatchability			Phosphorus Content of Yolk[5] (%)
	Winter	Summer	Total	Winter (%)	Summer (%)	Total (%)	Winter (%)	Summer (%)	Total (%)	Winter (%)	Summer (%)	Total (%)	
6	101	233	334	84.15	83.26	83.53	90.58	82.98	83.87	76.23	69.09	70.95	0.569±.020
7[2]	117	244	361	69.22	71.31	70.63	95.06	85.05	88.23	65.81	60.65	62.32	0.577±.004
8	115	253	368	88.69	91.69	90.76	93.13	89.22	90.71	82.60	81.81	82.33	0.585±.007
9[3]	116	226	342	68.10	77.87	74.56	91.13	83.52	85.88	62.06	65.04	64.03	0.575±.010
18[4]	132	268	400	68.93	66.04	67.00	80.21	88.70	85.82	55.30	58.58	57.50	0.584±.006

1 Four males placed in each pen on Feb. 5, 1958.

2 Male died May 9, 1958 -- not replaced.

3 Male died Apr. 3, 1958 -- not replaced.

4 Male died Feb. 2, and March 12, 1958 -- not replaced. Four males added May 13, 1958.

5 Ten eggs taken from each lot for analysis.

6 All averages are weighed.

Table 8a

ANALYSIS OF VARIANCE ON THE EFFECTS OF PHOSPHORUS SUPPLEMENTS ON THE HATCHABILITY OF FERTILE EGGS

Source of Variation	df	M.S.
Season	1	57.92
Period in Season	6	36.36
Treatment	4	72.39
Treatment x Season	4	18.09
Treatment x Period in Season	24	153.61

between treatments in any of the lots (Table 8a). The performance of the negative control is in agreement with the work of O'Rourke et al. (1954), who stated that 0.3 percent total phosphorus was sufficient to maintain "normal" hatchability.

The similarity in the hatchability of fertile eggs between the supplemented and non-supplemented lots can be further explained on the basis of the distribution of phosphorus in the yolk. The phosphorus content of the yolk was determined according to the method of Koenig and Johnson (1942). The concentration of this element was found to range from 100±5.3 to 108±3.2 mg. per yolk, regardless of dietary treatment. This level is similar to that observed by Romonoff and Romonoff (1949). The phosphorus content of the shell as shown by analysis ranged from 4.75 mg. to 5.41 mg. Information concerning the transfer of phosphorus from the shell to the embryo is not available. Since approximately 90 to 95 percent phosphorus of the egg was found in the yolk, it might be assumed that the yolk is the chief source of this element for the embryonic development. Since the phosphorus concentration of the yolks were similar, all lots contained sufficient phosphorus for normal hatchability.

Mortality

The highest mortality (26%) for the total experimental period was recorded in the lot receiving all dicalcium phosphate

(Lot 9, Table 9). The lot receiving no supplemental phosphorus (Lot 18) had the lowest mortality rate (16%).

Lot 9 had the lowest rate of mortality during the winter months (2%), but increased sharply during the summer months (24%). With the exception of Lots 8 and 18 there was a slight increase in mortality during the summer months. Post mortem diagnosis of birds at the University Pathology Laboratory, as evidenced from the causes summarized in Table 9, revealed that no deaths could be attributed to the dietary treatments. However, the lot receiving all dicalcium phosphate (Lot 9) appeared to have less resistance during the summer months.

Egg Weight

The largest eggs for the entire experimental period were produced by birds in Lots 7 and 9 (Table 10). These lots were supplemented with the higher levels of dicalcium phosphate, 14.80 and 21.30 gm/kg., respectively. They produced significantly heavier eggs ($P < .01$) than those in the negative control (Lot 18), and those receiving the higher levels of soft phosphate (Lots 6 and 8). Hens in the latter two lots received 45 and 27.6 gm./kg. of soft phosphate, respectively.

Information concerning the effect of phosphorus on the egg weight is very limited. Evans *et al.* (1943) were

Table 9

CAUSES AND DISTRIBUTION OF MORTALITY

Lot No.	Post-Mortem Diagnosis									Summary of Mortality		
	Eversion of Oviduct	Broken Yolks	Lympho-matosis	Tumors	Nephritis	Chronic Coryza	Viceral Lympho-mytosis	Hemorrhage	Unidentified Deaths	Winter (%)	Summer (%)	Total (%)
	Number of Birds											
6	2	6	--	1	--	--	1	1	2	8	14	22
7	1	3	2	--	--	--	2	--	2	10	14	24
8	4	3	--	--	1	1	--	--	1	10	10	20
9	4	3	2	1	--	--	--	--	3	2	26	28
18	2	2	--	--	1	--	--	--	3	8	8	16

Table 10

EFFECT OF PHOSPHORUS SUPPLEMENTS ON THE EGG WEIGHTS OF S. C. WHITE LEGHORN PULLETS

Lot No.	Supplements to Diet (gm./kg.)		Average Egg Weight		
	Soft Phos.	Dical. Phos.	Winter (gm.)	Summer (gm.)	Total (gm.)
6	45.00	-----	60.28	60.45	60.34
7	13.75	14.80	60.71	61.01	60.88**
8	27.50	8.30	59.99	59.51	59.72
9	-----	21.30	60.58	61.23	60.95**
18	-----	-----	60.02	60.28	60.17

** ($P < .01$)

ANALYSIS OF VARIANCE

Source of Variation	df	M.S.
Season	1	0.94
Period in Season	6	1.02
Treatments	4	2.05**
Treatment x Season	4	0.28
Treatment x Period in Season	24	0.19

** ($P < .01$)

unable to show any differences in egg weight by feeding laying hens graded levels of bone meal and defluorinated phosphate. It has been recognized for some time that heredity may influence egg weight (Farnsworth and Nordskoy, 1955; and Hicks, 1958). Other factors which may influence egg weight are the age of the bird, the seasonal temperature, and various nutritional factors. However, there is still some question as to the effect of the latter.

The mean average egg weights for winter and summer seasons are summarized in Table 10. The lots receiving the higher level of dicalcium phosphate (Lots 7 and 9) had significantly heavier ($P \leqslant .01$) eggs during the winter months than the other lots tested.

The egg weights during the summer season followed the same pattern as for the winter season. Hens in the lots receiving the higher levels of dicalcium phosphate continued to produce significantly heavier ($P \leqslant .01$) eggs during the summer season than the other lots tested.

Analysis of variance showed that the season had an effect on the weight of the egg. However, it should be pointed out that the season is affected by the age of the bird and the environmental temperature.

There was a small increase in egg weight during the summer season when the average weightfor the entire summer period was considered. It was reported by Rosenberg and Tanaka (1951) that normal environmental temperatures (80° F) did not affect egg weight. Heywang (1956) indicated that the high environmental temperatures (av. max. 101°F) in Arizona, caused a reduction in egg weight. Froning and Funk (1958) also reported that egg weight declined during the summer months when the maximum monthly temperatures were approximately 90°F. Huston (1958) reported that egg weight was not affected by "normal" environmental temperatures, but decreased slightly when the hens were subjected to a constant 90°F temperature. Since the data of the above investigators was presented on a monthly basis it was assumed that the differences between seasons could not be adequately demonstrated by grouping monthly egg weights into a winter and summer season as done here. However, when the average egg weight was compared on a monthly basis (Table 11) it can be seen that there was definitely no decline in egg weight associated with high environmental temperatures. There is presently no explanation for the fact that the egg weight in this experiment increased slightly during the summer months.

When the eggs were placed into the U.S.D.A. weight distribution market classes (Table 12), Lots receiving the higher levels of dicalcium phosphate (Lots 7 and 9) showed a significant increase ($P < .01$) in the percent of extra large eggs. These lots also produced significantly heavier ($P < .01$) eggs than the other lots tested. The lot receiving all soft phosphate showed a significant increase ($P < .01$) in the percent of large eggs. There was no significant

Table 11

RELATIONSHIP OF AVERAGE MONTHLY EGG WEIGHTS IN GRAMS TO THE MAXIMUM AND MINIMUM TEMPERATURES

Period Ending	Max. Temp.	Min. Temp.	Lot 6	Lot 7	Lot 8	Lot 9	Lot 18
Jan. 31	66.3	39.1	59.63	60.28	59.39	60.00	59.48
Feb. 28	71.5	46.5	59.93	60.67	59.64	60.38	59.62
Mar. 31	70.4	47.4	61.02	61.08	60.88	61.27	60.46
Apr. 30	79.9	52.5	61.04	61.84	59.72	61.49	60.44
May 31	95.3	69.1	60.43	61.15	58.74	60.82	59.85
June 30	102.2	70.5	59.95	61.08	59.46	51.52	60.30
July 31	98.2	77.1	60.07	61.10	59.75	60.84	60.09
Aug. 15	94.4	76.2	61.12	60.18	60.50	61.56	61.60

Table 12

THE EFFECT OF PHOSPHORUS SUPPLEMENTS ON THE AVERAGE PERCENTAGE DISTRIBUTION OF THE VARIOUS MARKET SIZES OF EGGS

Lot No.	Extra Large			Large			Medium			Small		
	Winter (%)	Summer (%)	Total (%)	Winter (%)	Summer (%)	Total (%)	Winter (%)	Summer (%)	Total (%)	Winter (%)	Summer (%)	Total (%)
6	24.33	24.86	24.71	59.03	61.43	60.53**	16.77	13.44	14.68	0.13	0.28	0.36
7	29.06	35.66	33.20**	54.73	47.36	50.13	16.23	16.62	16.67	0.00	0.40	0.24
8	23.03	25.04	24.30	57.00	55.92	56.33	19.07	18.10	18.46	0.73	0.92	0.91
9	29.76	31.26	30.71**	50.10	56.56	54.13	19.36	11.78	14.62	0.86	0.36	0.55
18	25.40	32.00	29.52	57.40	49.70	52.60	16.97	17.78	17.40	0.13	0.40	0.30

** ($P < .01$)

1 U.S.D.A. Agricultural Marketing Service Bulletin (1955).

Table 12a

ANALYSIS OF VARIANCE ON THE EFFECT OF PHOSPHORUS SUPPLEMENTS ON THE AVERAGE PERCENTAGE DISTRIBUTION OF THE VARIOUS MARKET SIZES OF EGGS

Source of Variation	Percent Extra Large		Percent Large		Percent Medium	
	df	M.S.	df	M.S.	df	M.S.
Season	1	111.19	1	20.38	1	42.74
Period in Season	6	73.50	6	15.84	6	38.72
Treatment	4	120.66**	4	114.58**	4	23.96
Treatment x Season	4	16.05	4	81.45	4	22.31
Treatment x Period in Season	24	12.40	24	7.23	24	26.81

** ($P < .01$)

difference ($P \geq .05$) in the medium weight class in any of the treatments. This indicates that the greatest weight change between lots appeared between the large and extra large weight classes.

Albumin Height and Haugh Index

The albumin heights of eggs for the total period in the lots receiving the higher levels of soft phosphate (Lots 6 and 8) and the lot receiving no supplemental phosphorus (Lot 18) were found to be significantly higher ($P < .01$) than those in the lots receiving the higher levels of dicalcium phosphate (Lots 7 and 9, Table 13). Lot 9 had a significantly higher ($P \leq .01$) albumin height than Lot 7. The performance of Lot 7 cannot be explained on the basis of dietary treatment. Evidently some factor other than the ones measured in this study affected the height of the albumin in this lot.

The lots receiving no dicalcium phosphate produced eggs with significant higher ($P \leq .01$) albumin heights during the winter season than the other lots tested.

There was a slight decrease (0.02 to 0.27 mm.) in the height of the albumin during the summer period in all lots. No information is available concerning the effect of supplemental phosphorus on albumin height. However, Froning and Funk (1958) reported that the albumin was higher during certain seasons of the year, but did not necessarily

Table 13

EFFECT OF PHOSPHORUS SUPPLEMENTS ON ALBUMIN HEIGHT AND HAUGH INDEX

Lot No.	Supplements to Basal Diet (gm./kg.)		Average Egg Weight			Average Albumin Height			Average Haugh Index		
	Soft Phos.	Dical. Phos.	Winter (gm.)	Summer (gm.)	Total (gm.)	Winter (mm.)	Summer (mm.)	Total (mm.)	Winter	Summer	Total
6	45.00	-----	60.28	60.45	60.34	6.32	6.10	6.18	77.85	76.34	76.91**
7	13.75	14.80	60.71	61.01	60.88**	5.94	5.76	5.83	74.99	73.36	73.96
8	27.50	8.30	59.99	59.51	59.72	6.13	6.11	6.12**	75.94	75.91	75.91
9	-----	21.30	60.58	61.23	60.95**	6.02	6.04	6.03	75.57	75.24	75.36
18	-----	-----	60.02	60.28	60.17	6.35	6.08	6.15**	77.81	76.99	77.29**

** ($P < .01$)

Table 13a

ANALYSIS OF VARIANCE FOR THE EFFECT OF PHOSPHORUS SUPPLEMENTS ON ALBUMIN HEIGHT AND HAUGH INDEX

Source of Variation	Albumin Height		Haugh Index	
	df	M.S.	df	M.S.
Season	1	0.120	1	6.99
Period in Season	6	0.077	6	5.24
Treatment	4	0.160**	4	13.86**
Treatment & Season	4	0.022	4	0.97
Treatment and Period in Season	24	0.009	24	0.89

** ($P < .01$)

parallel that of temperature.

The height of the albumin was suggested as a measure of the internal quality of an egg by Wilgus and Van Wagenen (1936). Albumin height as a measure of internal quality had only limited usefulness, since a change in albumin height from 10 to 9 mm. represented an unimportant difference in the quality of the egg, whereas a change from 3 to 2 mm. represented a noticeable quality change. Haugh (1937a) showed that a 40 gram egg with an albumin height of 4 mm. had a better internal quality than a 60 gram egg with the same albumin height. Haugh (1937b) further derived formulas that changed albumin height to interior quality units (Haugh Units), whose numerical value represented the "true" internal quality of the egg. These formulas compensated for the weight of the egg, that is, if the weight remained constant the internal quality varied as a logarithmic function of the albumin height.

The Haugh Units for the total periods are summarized in Table 13. The lots receiving no dicalcium phosphate (Lots 6 and 18) had a significantly higher ($P < .01$) Haugh Index than the other lots tested. This would indicate that, possibly, dicalcium phosphate has the undesirable quality of lowering the albumin height.

Analysis of variance indicated that the season had a significant effect ($P < .01$) on the Haugh Units. However, treatment--season interaction was found not to be significant

($P \geqslant .05$) indicating that the observed differences between groups were consistent from season to season (Table 13a). The Haugh Units during the winter months were significantly higher ($P < .01$) in Lots 6 and 18 than in the other lots tested. The difference between winter and summer seasons was small and was found not to be significant. When the Haugh Units were compared on a monthly basis (Table 14) no definite pattern could be established associating a change in the Haugh Units with environmental temperature. These results are in agreement with Froning and Funk (1958), who were unable to establish any definite relationship between the Haugh Units and maximum environmental temperatures. The effect of supplemental phosphorus on the Haugh Units has not been studied previously. Nevertheless, it should again be pointed out that the basal diet in this study contained a level of phosphorus too high to evaluate the effect of supplemental phosphorus on albumin height and Haugh Units, since Lot 18, receiving no supplemental phosphorus, produced results equal to those in the supplemented groups.

Using the Haugh Units, the eggs were placed in the U.S.D.A. market classes (Table 15). Lot 7, which had the lowest Haugh Units, also had significantly ($P \leqslant .01$) fewer AA eggs than the other lots tested. However, with the exception of Lot 7, there was no significant difference ($P \geqslant .05$) between any of the market classes. As stated

Table 14

RELATIONSHIP OF HAUGH UNITS TO
ENVIRONMENTAL TEMPERATURES

Period Ending	Max. Temp.	Min. Temp.	Lot 6	Lot 7	Lot 8	Lot 9	Lot 18
Jan. 31	66.3	39.1	76.53	74.08	75.48	74.14	76.75
Feb. 28	71.5	46.5	78.13	75.70	78.76	76.13	79.07
Mar. 31	70.4	47.4	78.91	75.20	73.48	76.45	77.61
Apr. 30	79.9	52.5	77.75	73.79	75.44	75.04	79.31
May 31	95.3	69.1	77.64	73.48	76.92	75.95	77.61
June 30	102.2	70.5	76.18	72.52	75.28	76.26	77.44
July 31	98.2	77.1	74.10	72.03	75.23	74.94	75.57
Aug. 15	94.4	76.2	76.04	75.00	76.66	74.00	75.92

Table 15

EFFECT OF PHOSPHORUS SUPPLEMENTS ON U.S.D.A. GRADE DISTRIBUTION

Lot No.	Phos. Supplement Added to Basal Diet (gm./kg.)		Average Percent "AA"			Average Percent "A"			Average Percent "B" and "C"		
	Soft Phos.	Dical. Phos.	Winter	Summer	Total	Winter	Summer	Total	Winter	Summer	Total
6	45.00	-----	51.6	40.8	44.9	48.0	57.0	53.7	0.3	2.0	1.4
7	13.75	14.80	32.3	30.3	31.1**	66.4	66.0	66.1**	1.1	3.8	2.5
8	27.50	8.30	41.6	41.9	41.8	58.6	57.1	57.7	0.1	1.0	0.7
9	-----	21.30	41.1	43.0	42.3	58.8	54.7	56.2	0.3	2.3	1.5
18	-----	-----	44.7	39.6	41.5	55.1	60.4	58.4	0.0	0.1	0.1

** ($P < .01$)

Table 15a

ANALYSIS OF VARIANCE OF THE EFFECT OF PHOSPHORUS SUPPLEMENTS ON U.S.D.A. GRADE DISTRIBUTION

Source of Variation	% AA		% A		% B & C	
	d f	M.S.	d f	M.S.	d f	M.S.
Season	1	91.88	1	21.98	1	20.79
Period in Season	6	80.96	6	61.32	6	3.59
Treatment	4	226.80**	4	172.19**	4	8.18
Treatment x Season	4	48.20	4	55.17	4	1.99
Treatment x Period in Season	24	22.62	24	20.93	24	3.96

** ($P < .01$)

previously, it is doubtful that the performance of Lot 7 is due to dietary treatment.

Mottling

There were no significant differences ($P > .05$) between treatments during the winter, summer, or total period, of eggs with no mottling (Table 16). Analysis of variance indicated that there was a highly significant decrease ($P < .01$) in the mottling of the yolk during the summer months, ranging from 6.4 to 12.8 percent. The largest percentage of mottled yolks were placed in the 1-2 scoring range. These eggs had very little mottling. Only a small percentage of the eggs were placed in 3-5 and 6-10 scoring classes. The total mottling for these two classes ranged from 3.7 to 6.7 percent. No information is available concerning the effect of phosphorus supplements on the mottling of the yolk. However, nutritional additives such as Nicarbazin® have been shown to increase the incidence of yolk mottling (Polin *et al.*, 1957).

Blood Spots

The occurrence of blood spots are summarized in Table 17. A significantly larger ($P < .01$) number of blood spots were found in the lots receiving dicalcium phosphate (Lots 7, 8, and 9). This was evidenced during the winter,

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® 4,4' - dinitrocarbanilide with 4,6-dimethylpyrimidine.

Table 16

EFFECT OF PHOSPHORUS SUPPLEMENTS ON AVERAGE EGG YOLK MOTTLING OF EGGS FROM S. C. WHITE LEGHORN PULLETS

Lot No.	Supplements to Basal Diet(gm/kg)		0 Mottling			1-2 Mottling			3-5 Mottling			6-10 Mottling		
	Soft Phos.	Dical. Phos.	Winter (%)	Summer (%)	Total (%)	Winter (%)	Summer (%)	Total (%)	Winter (%)	Summer (%)	Total (%)	Winter (%)	Summer (%)	Total (%)
6	45.00	-----	73.6	80.0	77.6	16.8	15.1	15.7	7.9	4.1	5.5	1.9	1.3	1.2
7	13.75	14.80	75.8	86.2	82.3	17.4	11.0	13.4	4.7	1.9	3.0	2.3	0.8	1.4
8	27.50	8.30	72.9	85.7	80.9	21.0	18.6	14.9	5.4	2.1	3.4	0.7	0.5	0.6
9	-----	21.30	79.2	85.9	83.4	15.2	10.2	12.1	4.6	3.2	3.8	1.0	0.7	0.8
18	-----	-----	77.0	85.3	82.2	18.2	11.7	14.1	3.8	3.2	3.0	1.1	0.5	0.7

Table 16a

ANALYSIS OF VARIANCE ON THE EFFECTS OF PHOSPHORUS SUPPLEMENTS ON PERCENT "O" MOTTLING

Source of Variation	df	M.S.
Season	1	750.17
Period in Season	6	48.81
Treatments	4	39.57
Treatment x Season	4	14.19
Treatment x Period in Season	24	23.01

Table 17

EFFECT OF PHOSPHORUS SUPPLEMENTS ON THE OCCURRENCE OF BLOOD SPOTS IN EGGS FROM S. C. WHITE LEGHORN PULLETS

Lot No.	Supplements to Basal Diet(gm/kg)		Average of Total Blood Spots			Average Large Spots			Average Small Spots		
	Soft Phos.	Dical. Phos.	Winter (%)	Summer (%)	Total (%)	Winter (%)	Summer (%)	Total (%)	Winter (%)	Summer (%)	Total (%)
6	45.00	-----	9.0	6.9	7.7**	48.5	27.0	35.1	51.5	73.0	64.9
7	13.75	14.80	12.8	10.1	11.1	65.3	38.6	48.6	34.7	61.4	51.4
8	27.50	8.30	13.3	8.3	10.4	62.0	30.9	42.6	38.0	69.1	57.4
9	-----	21.30	10.8	7.8	8.9	56.1	22.0	34.8	43.9	78.0	65.2
18	-----	-----	7.8	5.9	6.6**	41.3	27.7	32.8	58.8	72.3	67.2

** ($P < .01$)

Table 17a

ANALYSIS OF VARIANCE ON THE EFFECT OF PHOSPHORUS SUPPLEMENTS ON TOTAL BLOOD SPOTS

Source of Variation	df	M.S.
Season	1	75.36
Periods in Season	6	16.24
Treatment	4	28.03**
Treatment x Season	4	1.27
Treatment x Periods in Season	24	5.68

** $(P < .01)$

summer, and for the total period. During the winter season the largest number of total spots were large spots, while during the summer season the large spots decreased, but the small spots (less than 1/8 inch in diameter) showed a marked increase. Since no information is available concerning the effect of supplemental phosphorus on the occurrence of blood spots, the reason for the performance of Lots 6 and 18 cannot be postulated. However, it could be possible that the presence of dicalcium phosphate in the diets increases the incidence of blood spots in the yolks. Lerner and Smith (1942) found a definite increase in the incidence of blood spots after April 1. However, Jeffery (1945) reported the highest incidence of blood spots at the beginning of the laying year, and a decrease through August, while Froning and Funk (1958) reported the highest incidence of blood spots in March.

CHAPTER II RESULTS AND DISCUSSION: THE EFFECT OF PHOSPHORUS SUPPLEMENTS ON THE EXTERNAL QUALITY AND THE CHEMICAL COMPOSITION OF THE SHELL

The previous discussion has been concerned with the effect of phosphorus supplements on the rate of egg production, and on the internal quality of the egg. The height of the albumin, the Haugh Index, U.S.D.A. quality grades, yolk mottling, and blood spots were used as measures of the internal quality of the egg. The discussion to follow will deal with the effect of the phosphorus supplements on the quality of the shells of the same eggs.

The criteria for measuring the relative strength of the shell vary. Evans _et al._ (1943) used absolute shell weight, and the thickness of the shell expressed in mm., to determine the quality of the shell, while the porosity of the shell was used by Almquist (1934). Hendricks _et al._ (1931) demonstrated that the quality of the shell is a function of the shell index and is proportional to the weight per unit area of egg. In this study the weight of the shell, percent shell, and the shell index were used as measures of the shell quality.

The differences in the absolute weights of the shells between treatments were relatively small, ranging from .01 to .07 gm (Table 18) for the entire experimental period. The average weight of the shells ranged from 5.12 gm.

Table 18

EFFECT OF PHOSPHORUS SUPPLEMENTS ON EGG WEIGHT[1], SHELL WEIGHTS[1], AND PERCENT SHELL[1], OF S.C. WHITE LEGHORN PULLETS

Lot No.	Supplements to Basal Diet (gm./kg.)		Average Egg Weight			Average Shell Weight			Average Percent Shell		
	Soft Phos.	Dical. Phos.	Winter (gm.)	Summer (gm.)	Total (gm.)	Winter (gm.)	Summer (gm.)	Total (gm.)	Winter (gm.)	Summer (gm.)	Total (gm.)
6	45.00	-----	60.28	60.45	60.34	5.31	4.98	5.12	8.42	9.24	8.46
7	13.75	14.80	60.71	61.01	60.88[2]	5.34	5.00	5.15	8.79	8.20	8.45
8	27.50	4.30	59.99	59.51	59.72	5.75	4.92	5.14	8.92	8.37[4]	8.61[4]
9	-----	21.30	60.58	61.23	60.95[2]	5.37	5.05[4]	5.19[3]	8.87	8.25	8.52
18	-----	-----	60.02	60.28	60.17	5.35	5.06[4]	5.18[3]	8.92	8.39[4]	8.61[4]

1 Weighted averages.

2 ($P < .01$).

3 ($P < .05$) when compared with Lot 6.

4 ($P < .05$) when compared with Lots 6 and 7.

Table 18a

ANALYSIS OF VARIANCE FOR THE EFFECTS OF PHOSPHORUS SUPPLEMENTS ON SHELL WEIGHT, PERCENT SHELL, AND SHELL INDEX OF EGGS FROM S. C. WHITE LEGHORN PULLETS

Source of Variation	Shell Weight		Percent Shell		Shell Index	
	df	M.S.	df	M.S.	df	M.S.
Season	1	1.140	1	3.360	1	0.006600
Period in Season	6	0.067	6	.012	6	0.000316
Treatments	4	0.013*	4	.045*	4	0.000075**
Treatment x Season	4	0.003	4	.000	4	0.000000
Treatment x Period in Season	24	0.003	24	.011	24	0.000017

* ($P < .05$)

** ($P < .01$)

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for the eggs produced by hens in Lot 6 receiving all soft phosphate, to 5.19 gm. for those produced by hens fed dicalcium phosphate as the only source of supplemental phosphorus (Lot 9). The shells in the latter lot as well as those in Lot 18, fed no supplemental phosphorus, were significantly heavier ($P \leqslant .05$) than those in Lot 6 (Table 18). No difference was noted among the weights of shells in Lot 9 and those in Lot 18. The weight of shells of eggs from lots fed 13.75 gm./kg. (Lot 7) and 27.50 gm./kg. (Lot 8) of soft phosphate, were not significantly different ($P \geqslant .05$) from those in Lot 6, having the heaviest egg weight.

Although statistical differences were observed among treatments (Table 18a), these differences were so small that it is doubtful the quality of the shell would be affected appreciably. The lot receiving no supplemental phosphorus (Lot 18) and those receiving the higher levels of soft phosphate (Lots 6 and 8) had significantly smaller ($P \leqslant .01$) eggs than the lots receiving the higher levels of dicalcium phosphate (Lots 7 and 9). However, when the shell weights are considered no such similar pattern could be established. Lot 9 had the highest egg weight and also the highest shell weight, but in Lot 7 the shell was not as heavy proportionately as in Lot 9.

Analysis of variance indicated that season had a highly significant effect ($P \leqslant .01$) on the weight of the shell (Table 18a). Although the maximum difference in the average shell weight between treatments during the winter season was only 0.06 grams, the hens fed all dicalcium phosphate (Lot 9) were found to have significantly heavier ($P < .05$) shells than eggs from hens fed soft phosphate as the only source of supplemental phosphorus.

Egg shell weight declined sharply in all lots during the summer months. This decline ranged from 0.31 to 0.37 grams. The lot receiving no supplemental phosphorus (Lot 18) had the least decline. Lot 9, supplemented with only dicalcium phosphate, and Lot 18, receiving no supplemental phosphorus had significantly heavier ($P < .05$) shells during the summer months when compared with the lots receiving the higher levels of soft phosphate (Lots 6 and 8). Lot 9 had the highest egg weight for the summer months, and also maintained a high shell weight for that period, whereas Lot 7, supplemented with 13.75 gm./kg. of soft phosphate and 14.80 gm./kg. dicalcium phosphate, had a high egg weight, but did not maintain a shell proportional to its weight.

The decline in the absolute weight of the shell as associated with a change in environmental temperature is shown in Table 19. It can be seen that the shell weight remained relatively constant during the winter period (January through April). The first apparent decline in shell weight was noted during the month of April, when the mean maximum temperature increased to 79.8°F. This, as

Table 19

RELATIONSHIP OF MONTHLY SHELL WEIGHTS TO MAXIMUM AND MINIMUM LAYING HOUSE TEMPERATURES

Period Ending	Av. Monthly Temperatures		Lot 6	Lot 7	Lot 8	Lot 9	Lot 18
	Max.	Min.					
January 31	66.3	39.1	5.28	5.32	5.31	5.41	5.39
February 28	71.5	46.5	5.32	5.30	5.39	5.34	5.32
March 31	70.4	47.4	5.34	5.39	5.35	5.38	5.36
April 30	79.9	52.5	5.21	5.27	5.22	5.21	5.20
May 31	95.3	69.1	5.01	5.01	5.00	5.03	5.01
June 30	102.2	70.5	4.92	4.93	4.93	5.03	5.04
July 31	98.2	77.1	4.78	4.81	4.75	4.88	4.95
August 15	94.4	76.2	4.85	4.86	4.91	5.02	5.13

stated earlier, was chosen as the beginning of the summer season. Shell weight continued to decline in all lots until the eighth experimental period. During this period the shell weight increased slightly in all lots over that of the preceding month. It is doubtful, however, that the increase can be explained on the basis of temperature, since the maximum temperature for this period was 94.4°F.

Information pertaining to the effect of phosphorus supplements on the weight of egg shells is very limited. Evans et al. (1944a) were unable to show any difference in the absolute weight of the egg shells by feeding laying hens graded levels of defluorinated phosphate and bone meal. However, these investigators did observe that the weight of the shell decreased as the experiment progressed from December to August. Wilhelm (1940) also reported that the weight of the egg shell decreased during the summer months.

Percent Shell

When the average percent shell is considered for the total periods, the lot receiving no supplemental phosphorus (Lot 18, Table 20) had significantly higher ($P < .05$) percent shell than did the lot supplemented with all soft phosphate (Lot 6), and the lot supplemented with 13.75 gm./kg. soft phosphate and 14.83 gm./kg. dicalcium phosphate (Lot 7). The lot receiving all dicalcium phosphate was not significantly different ($P > .05$) from either population (Lot 9).

Table 20

THE EFFECT OF PHOSPHORUS SUPPLEMENTS ON PERCENT SHELL AND SHELL INDEX OF EGG SHELLS FROM S. C. WHITE LEGHORN PULLETS

Lot No.	Supplements to Basal Diet (gm./kg.)		Average Percent Shell			Average Shell Index		
	Soft Phos.	Dical. Phos.	Winter	Summer	Total	Winter	Summer	Total
6	45.00	-----	8.82	8.24	8.46	0.3660	0.3398	0.3496
7	13.75	14.80	8.79	8.21	8.45	0.3677	0.3404	0.3506
8	27.50	8.30	8.92	8.37*	8.61*	0.3720	0.3442	0.3546
9	-----	21.30	8.87	8.25	8.52	0.3710	0.3426	0.3533
18	-----	-----	8.92	8.39*	8.61*	0.3720	0.3486**	0.3574**

* ($P < .05$) when compared with Lots 6 and 7.

** ($P < .01$) when compared with Lots 6 and 7.

It should be noted that Lot 9 had the heaviest shells, but when considered on a relative basis the same pattern was not followed. The reason for this decline was probably due to the fact that this lot had the heaviest eggs.

There was no significant differences ($P \geq .05$) between treatments during the winter season. During the summer season there was a sharp decline in the percent shell in all lots, ranging from 0.53 to 0.62 percent. This decline was highly significant in ($P \leq .01$) all lots. It should be noted that the lot receiving all dicalcium phosphate (Lot 9) had a greater absolute decline than the other lots tested. During the summer season Lots 8 and 18 had a significantly higher percentage ($P \leq .05$) shell when compared with Lots 6 and 7. Since there were no differences between treatments during the winter season, all the differences for the entire period were due to those occurring during the summer months.

The relationship between shell percentage and the environmental temperatures are shown in Table 21. As with the shell weight, the monthly shell percentages remained relatively constant for the winter period (January through March). A definite decline in the percent shell was observed in April, and continued to decline until the last experimental month (August). During August the percent shell followed the same pattern as did the shell weight. All lots increased slightly during this period over that of the preceding month.

Table 21

RELATIONSHIP OF MONTHLY SHELL PERCENTAGES TO MAXIMUM AND MINIMUM LAYING HOUSE TEMPERATURES

Period Ending	Av. Monthly Temperatures		Lot 6	Lot 7	Lot 8	Lot 9	Lot 18
	Max.	Min.					
January 31	66.3	39.1	8.8	8.8	8.9	9.0	9.1
February 28	71.5	46.5	8.9	8.7	9.0	8.8	8.9
March 31	71.4	47.4	8.8	8.8	8.8	8.8	8.9
April 30	79.9	52.5	8.5	8.5	8.7	8.5	8.6
May 31	95.3	69.1	8.3	8.2	8.5	8.3	8.4
June 30	102.2	70.5	8.2	8.1	8.3	8.2	8.4
July 31	98.2	77.1	8.0	8.0	7.9	8.0	8.2
August 15	94.4	76.2	8.2	8.1	8.1	8.2	8.3

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The results in this study parallel those of Miller and Bearse (1934), who showed that the shell percentage of eggs decreased consistently, beginning with March and continuing throughout the experiment. Wilhelm (1939) showed that the shell percentage decreased as the experiment progressed, but did not parallel that of shell weight. This would be expected, since Taylor and Lerner (1939) observed that egg weight had a greater influence on the percentage of shell than did the whell weight. This could possibly account for the higher percent shell found in Lots 8 and 18 of this study (Table 18), since hens in both of these lots produced smaller eggs than any of the other lots.

Shell Index

Another method for measuring shell quality, employed in this study, was that of Shell Index. It has been a common practice to compute as a measure of shell thickness, the number of grams of shell per unit weight of egg. However, Hendricks *et al.* (1931), in deriving a formula for the Shell Index, expressed the area as a function of the weight of the contents of the egg, rather than as a function of the weight of the entire egg.

$$I = \frac{\text{Weight of shell}}{(\text{Weight of the content of the egg})^{2/3}}$$

By using the above formula a shell thickness index is obtained, which is proportional to the weight of shell per unit area of egg.

The average shell indices for the entire study are summarized in Table 20. The lot receiving no supplemental phosphorus (Lot 18) followed the same pattern as for percent shell, that is, this lot had the highest percent shell and also the highest shell index. The average shell index for this lot was significantly higher ($P \leq .01$) when compared with the lot receiving all soft phosphate and the lot supplemented with 13.75 gm./kg. soft phosphate and 14.80 gm./kg. dicalcium phosphate (Lot 7). Lots 8 and 18 had a significantly higher ($P \leq .05$) percent shell, but only the lot receiving no supplemental phosphorus (Lot 18) had a higher shell index.

There was no significant difference ($P \geq .05$) in the shell index during the winter months. There was, however, a sharp decline in the shell index during the summer season, ranging from 2.34×10^{-2} to 3.8×10^{-2} units. The largest decline occurred in the lot receiving all soft phosphate (Lot 6). During the summer months the lot receiving no supplemental phosphorus (Lot 18) had a significantly higher ($P \leq .05$) shell index when compared to Lots 6 and 7. The lot receiving 27.5 gm./kg. soft phosphate and 8.3 gm./kg. dicalcium phosphate, and the lot receiving all dicalcium phosphate, were not significantly different from either population. When the shell indices were compared on a monthly basis (Table 22), no decline was observed until the month of April. This followed the same

Table 22

RELATIONSHIP OF MONTHLY SHELL INDICES TO MAXIMUM AND MINIMUM LAYING HOUSE TEMPERATURES

Period Ending	Av. Monthly Temperatures Max.	Min.	Lot 6	Lot 7	Lot 8	Lot 9	Lot 18
January 31	66.3	39.1	.362	.368	.371	.376	.377
February 28	71.5	46.5	.370	.365	.377	.369	.369
March 31	70.4	47.4	.366	.370	.368	.368	.370
April 30	79.9	52.6	.356	.358	.363	.354	.359
May 31	95.5	69.1	.344	.342	.351	.344	.347
June 30	102.2	70.5	.340	.336	.343	.341	.347
July 31	98.2	77.1	.329	.331	.328	.334	.342
August 15	94.4	76.2	.330	.335	.336	.340	.348

pattern as for shell weight and shell percent. All lots showed a slight increase in the shell index during the month of August.

It should be noted that Lot 18, receiving no supplemental phosphorus, had a significantly higher ($P < .05$) shell weight for the total period when compared with Lot 6, and a significantly higher ($P < .05$) percent shell when compared with Lots 6 and 7. This lot also had a significantly higher ($P < .01$) shell index when compared with Lots 6 and 7. The shell index minimizes the effect of the weight of the egg. No other lot tested gave superior results when all three of the above measurements were used to measure the quality of the shell.

It should be pointed out again that the basal diet in this study was not suitable for measuring the effect of supplemental phosphorus on the shell weight, shell percent, or shell indices, since a deficiency was not produced. The performance of the lot receiving no supplemental phosphorus (Lot 18) was equal to or superior to that of the supplemented lots. It is also possible that supplemental phosphorus has no effect on the above mentioned measurements, since in most cases, when significant differences were observed in the negative control, they were also observed in the supplemented lots.

Chemical Analysis of the Constituents of the Shell

The shells from the previous studies were individually weighed, pooled, and retained for chemical analysis, to determine if there was any difference in the mineral constituents of the shell due to dietary treatment. Calcium and phosphorus were present in the shell in relatively high concentrations, and magnesium, manganese, and iron, in trace amounts. The organic portion of the shell consisted almost entirely of proteinaceous materials. Since calcium and phosphorus were the two minerals present in large amounts in the shell, and the organic matter consisted mostly of protein, the relative concentration of these three constituents was determined.

The average phosphorus, calcium, and protein concentrations for the total period are summarized in Table 23. The lots receiving no soft phosphate (Lots 9 and 18) had significantly lower ($P \leq .01$) phosphorus concentrations than the lots receiving soft phosphate (Lots 6, 7, and 8). The average phosphorus concentration for the lots receiving soft phosphate was 1090 ppm., while the average phosphorus concentration for the lots receiving no soft phosphate was 1034 ppm. The differences between treatments were observed the first month in which analyses were made (Table 24), and these differences remained relatively constant throughout the course of the study.

Although there was a wide monthly variation in the calcium concentration (Table 25), the average calcium concentration for the total period remained relatively constant.

Table 23

EFFECT OF PHOSPHORUS SUPPLEMENTS ON THE TOTAL PROTEIN, PHOSPHORUS, AND CALCIUM CONTENT OF EGG SHELLS OF S. C. WHITE LEGHORN PULLETS

Lot No.	Supplements to Diet (gm./kg.)		Average Phosphorus Total Period (ppm.)	Average Calcium Total Period (%)	Average Protein Total Period (%)
	Soft Phos.	Dical. Phos.			
6	45.00	-----	1100**	36.76	4.62
7	13.75	14.80	1080**	36.82	4.56
8	27.50	8.30	1090**	36.84	4.64
9	-----	21.30	1040	36.85	4.85**
18	-----	-----	1028	36.85	4.59

** $(P < .01)$

Table 23a

THE EFFECTS OF PHOSPHORUS SUPPLEMENTS ON THE PROTEIN AND PHOSPHORUS CONTENT OF EGG SHELLS FROM S. C. WHITE LEGHORN PULLETS

Source of Variation	Protein		Phosphorus	
	df	M.S.	df	M.S.
Period	7	0.073	7	9,890
Treatments	4	0.100**	4	19,803**
Treatment x Period	28	0.049	28	1,085
Sample Error	96	0.027	90	575

** ($P \leqslant .01$)

Table 24

EFFECTS OF PHOSPHORUS SUPPLEMENTS ON THE PHOSPHORUS CONTENT OF EGG SHELLS FROM S. C. WHITE LEGHORN PULLETS

Period Ending	Phosphorus[1] (ppm.)				
	Lot 6	Lot 7	Lot 8	Lot 9	Lot 18
February 28	1171	1112	1140	1107	1104
April 1	1139	1125	1176	1081	1122
May 1	1136	1155	1147	1040	1122
June 1	1136	1113	1136	1106	1017
June 15	1099	1111	1100	1052	1030
July 1	1058	1029	1032	1001	929
August 1	1054	1038	1054	965	985
August 15	1003	956	934	964	915

1 Moisture free.

Table 25

THE EFFECT OF PHOSPHORUS SUPPLEMENTS ON THE CALCIUM CONTENT OF EGG SHELLS FROM S. C. WHITE LEGHORN PULLETS

Period Ending	Percent Calcium [1]				
	Lot 6	Lot 7	Lot 8	Lot 9	Lot 18
February 28	37.11	36.96	36.98	37.12	36.72
April 1	36.80	36.70	36.59	36.64	36.88
May 1	36.65	36.90	36.47	36.71	36.77
June 1	37.38	37.58	37.60	37.31	37.18
June 15	36.75	36.74	36.70	36.72	36.66
July 1	36.59	36.61	36.82	36.69	36.91
August 1	36.42	36.66	36.68	36.69	36.75
August 15	36.39	36.39	36.88	36.89	36.91

1 Moisture free.

The maximum difference in calcium concentrations between treatments for the entire experimental period was 0.09 percent.

The lot receiving all dicalcium phosphate (Lot 9) had a significantly higher ($P \leqslant .01$) shell protein content than the other lots tested (Table 23). Statistical analysis (Table 23a) indicates that there was a significant treatment by period interaction ($P \leqslant .05$); therefore, all of the differences observed among treatments were not consistent from season to season. The average protein content for Lot 9 was 4.85 percent, while the average for the other lots tested was 4.60 percent, as shown in Table 26. No work is available concerning the effect of phosphorus supplements on the mineral composition of the shell.

Statistical analysis indicates that the periods had a highly significant effect ($P \leqslant .01$) on the phosphorus concentration. This effect can best be shown by Figure 2. The monthly phosphorus concentrations varied greatly, but there was still a definite decline in all lots as the study progressed, regardless of dietary treatment. However, regression analysis indicates a significant difference in the rate of decline of the shell phosphorus between the supplemented and non-supplemented lots. The average phosphorus concentration in the supplemented lots (Lots 6, 7, 8, and 9) ranged from 1132 to 964 ppm., whereas for the lot receiving no supplemental phosphorus (Lot 18) the phosphorus concentration ranged from 1104 to 915 ppm. The rate of decline as shown by the regression

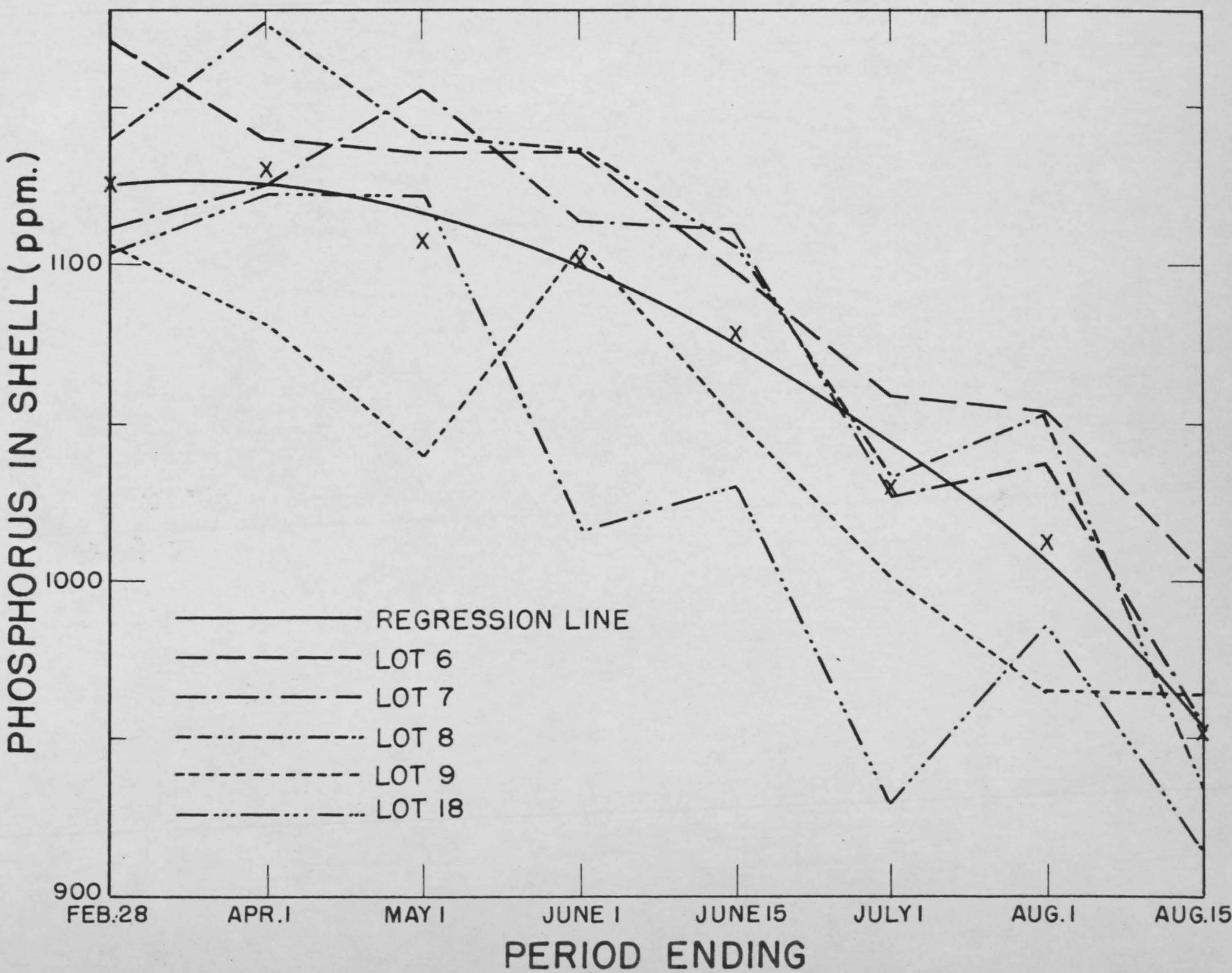

Fig. 2—Rate of decline of shell phosphorus in relation to experimental periods.

Table 26

PROTEIN[1] CONTENT OF EGG SHELLS

Period Ending	Total Protein (%)				
	Lot 6	Lot 7	Lot 8	Lot 9	Lot 18
February 28	4.94	4.74	4.54	5.24	4.89
April 1	4.46	4.24	4.12	5.25	4.83
May 1	4.54	4.35	4.47	4.67	4.37
June 1	4.48	4.92	4.93	4.80	4.49
June 15	4.59	4.43	4.82	4.68	4.24
July 1	4.61	4.77	4.64	4.66	4.83
August	4.65	4.43	4.86	4.73	4.49
August 15	4.65	4.60	4.72	4.77	4.57

1 N x 6.25 (moisture free).

curve in Figure 2 was curvilinear, being accelerated as the study progressed.

The calcium remained constant throughout the study (Table 25). This was indicated by the fact that the Ca:P ratios increased from the beginning to the end of the study (Table 27). As would be expected, the lot receiving no supplemental phosphorus (Lot 18) had a slightly higher Ca:P ratio than the supplemented lots. The average Ca:P ratios of the shells from hens in the supplemented lots ranged from 327.2 to 380.2, while the Ca:P ratios of shells from hens receiving no supplemental phosphorus ranged from 332.6 to 403.3.

The percentage protein in the shell dropped slightly in the lot receiving all dicalcium phosphate (Lot 9, Table 26). However, it should also be pointed out that this lot also had the highest percentage protein during the first two experimental periods. Although there was a decline in this lot, the protein concentration did not fall below that of the other lots. All other lots (Lots 6, 7, 8, and 18) remained relatively constant throughout the study.

It had been assumed that the mineral constituents of the shell remained constant regardless of the absolute or relative weight of the shell (Buckner <u>et al.</u>, 1923). Almquist (1934), in discussing thin and thick egg shells, concluded that the mineral constituents of the shell remained constant.

Table 27

EFFECTS OF PHOSPHORUS SUPPLEMENTS ON
THE CALCIUM--PHOSPHORUS RATIO OF EGG SHELLS
FROM S. C. WHITE LEGHORN PULLETS

Period Ending	Total Calcium (%)				
	Lot 6	Lot 7	Lot 8	Lot 9	Lot 18
February 28	316.9	332.4	324.4	335.3	332.6
April 1	323.1	326.2	310.1	338.9	328.7
May 1	324.4	319.4	319.6	352.9	327.7
June 1	329.0	337.6	330.9	337.3	365.6
June 15	334.4	330.7	333.6	349.0	355.9
July 1	345.8	355.8	356.8	366.5	377.3
August 1	345.5	353.2	348.0	380.2	373.1
August 15	362.8	380.6	394.9	382.7	403.3

However, when an average was taken of the phosphorus concentrations in the thick and thin shells, the concentrations were found to be 1270 and 1000 ppm. respectively. Futher analysis of the work of Common (1936) shows that when a thin shell egg shell was induced experimentally by withholding the calcium, the absolute and relative concentration of phosphorus decreased.

Since the shell weight, percent shell, and shell index all declined consistently as the study progressed, an attempt was made to correlate the declinein the relative weight of the shell with the decline in phosphorus concentration. Regression lines relating the phosphorus content of the shell with the relative weight of the shell is given in Figure 3. Regression analysis showed that the decline in phosphorus could be correlated with the decline in the relative weight of the shell (Table 28). The correlation values ranged from .77 to .79. No correlation could be found between the decline in the relative weight of the shell and the calcium and nitrogen content, further indicating that the calcium and phosphorus content remained relatively constant.

An attempt was made to physiologically associate this decline in phosphorus content of the shell with the relative weight of the shell (Table 29). Shells from eggs collected during the ninth experimental month were analyzed individually. The relative weight of the shell ranged from 6.08 to 9.88 percent.

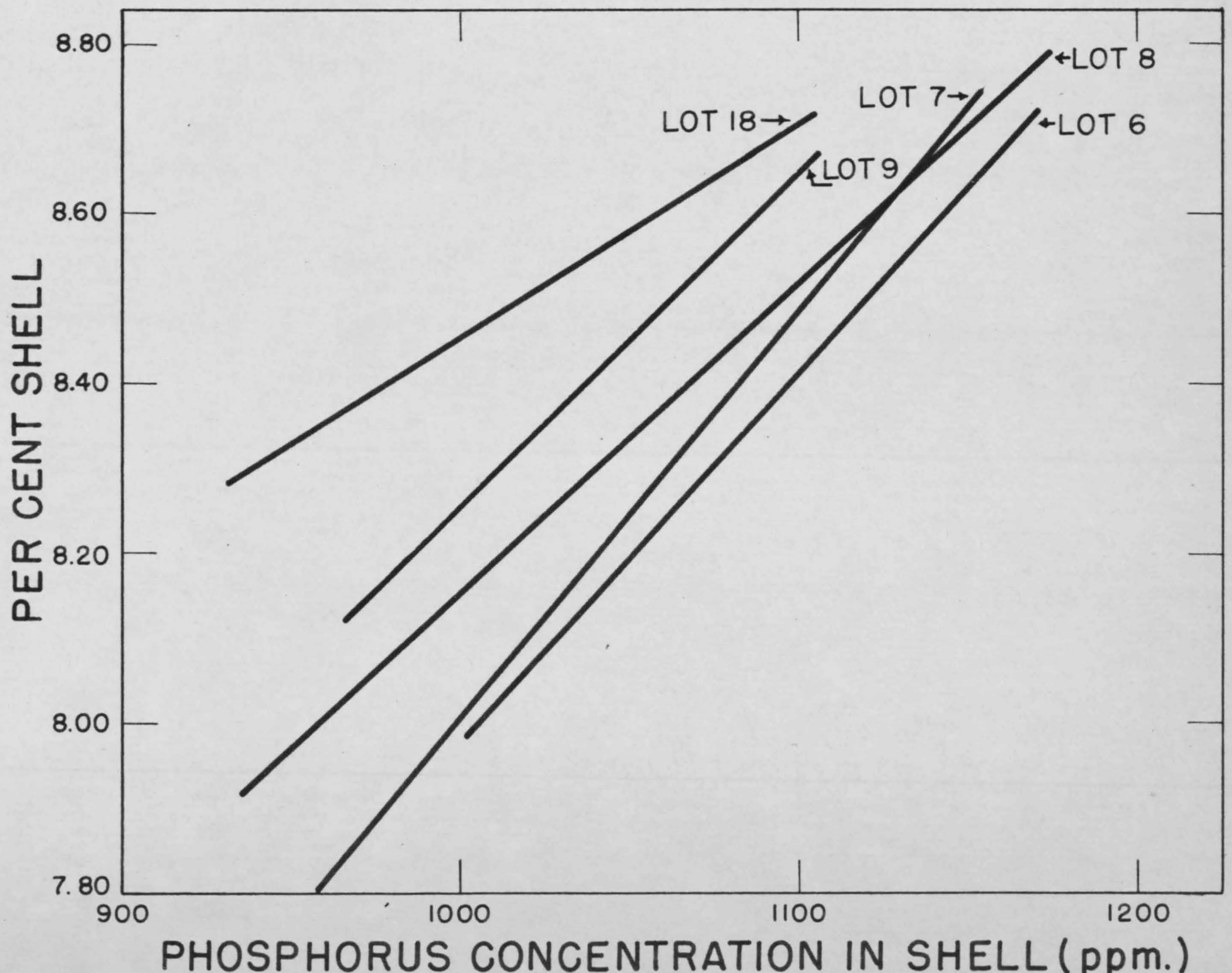

Fig. 3—Relationship of phosphorus concentration in the shell to percent shell.

Table 28

REGRESSION AND CORRELATION COEFFICIENTS FOR CALCIUM, PHOSPHORUS, AND PROTEIN ON PERCENT SHELL

	Regression Coefficients		Correlation Coefficients		
Lot No.	b Phos./ Shell	bx10^{-3} Shell/ Phos.	Phos.[1]-- % Shell	Ca.-- % Shell	Protein-- % Shell
6	146	4.34	.79	.49	.22
7	161	5.13	.90	.13	.39
8	167	3.58	.77	.01	.20
9	154	3.83	.82	.11	.81
18	261	2.58	.82	.28	.58

1 ($P < .05$)

Table 29

EGG WEIGHT, SHELL WEIGHT, PERCENT SHELL, AND CALCIUM AND PHOSPHORUS CONTENT OF INDIVIDUAL EGGS

Egg Wt. (gm)	Shell Wt. (gm)	Shell (%)	Phos. (ppm)	Calcium (%)	Egg Wt. (gm)	Shell Wt. (gm)	Shell (%)	Phos. (ppm)	Calcium (%)
68.9	4.95	7.18	1329	36.59	59.6	4.52	7.58	917	36.48
66.4	5.94	8.94	1019	36.95	59.6	4.34	7.23	1079	36.57
65.9	6.33	9.60	1193	36.87	59.4	4.75	7.99	1210	36.39
65.8	5.10	7.75	834	36.58	59.3	4.43	7.47	754	36.65
65.1	4.53	6.96	954	36.81	58.7	5.16	8.79	1019	37.06
64.9	5.31	8.18	1321	36.10	58.7	4.91	8.36	1213	37.01
64.4	5.12	7.95	687	37.21	58.7	5.22	8.89	1007	37.13
64.2	5.34	8.31	1012	37.27	58.0	3.53	6.08	912	36.06
62.0	5.61	8.99	912	36.62	57.5	4.71	8.15	928	36.81
61.7	5.89	9.54	951	36.73	57.4	4.78	8.32	955	36.62
61.7	4.09	6.63	1023	36.67	57.4	3.78	6.58	1006	36.56
61.4	5.29	8.62	910	36.90	56.9	5.18	9.10	1057	37.23
61.4	5.20	8.46	804	37.20	56.7	5.00	8.82	543	36.63
60.8	4.63	7.61	1123	36.96	53.7	4.52	8.41	1067	37.01
60.7	5.57	9.18	1201	36.83	53.0	3.94	7.43	851	36.81
59.8	5.33	8.91	817	36.48	50.1	4.95	9.88	1136	37.06
59.8	4.68	7.82	955	36.10	49.3	4.12	8.35	509	37.17

When phosphorus was determined, no definite correlation could be made between the percent shell and the phosphorus content of the shell, however, further investigation of this phase of the study will be pursued. It is possible that the method used in this study for phosphorus determinations is not sensitive enough to detect such small differences when the shells are analyzed individually.

No explanation is available for the decline in the phosphorus concentration observed in this study. As the phosphorus concentration declined, the environmental temperatures increased. However, the hens were also increasing in age. It has been shown that the shell gets thinner as the temperature increases (Wilhelm, 1940). However, at the present time the shell seems to be physiologically unrelated to the decline in phosphorus concentration. Further investigation will begin at this University soon, to check out the relationships and discrepancies found in this study.

Analysis of Membrane

Since a significant difference ($P \leqslant .01$) was observed between treatments in the phosphorus concentrations of the shell, it was found desirable to separate the shell from the membrane and determine the calcium, phosphorus, and protein concentrations of each component. Since much difficulty was encountered in the manual separation of the shell from the membrane, this was done only once during the course of study.

The relative concentrationsof the above mentioned elements found in the membrane are outlined in Table 30. The phosphorus concentration in the membrane ranged from 121 to 126 ppm, and that of calcium from 0.066 to 0.069 percent. It can be seen that most of the shell membrane is composed of protein, which constitutes from 96.54 to 97.89 percent of the total membrane. All the analyses were made on a moisture free basis.

Only the relative concentrations were determined because of the difficulty in removing the entire membrane from the shell. This author observed that after peeling away as much of the membrane as was possible from the shell, there always remained a coarse meshwork of fibers attached to the shell proper. A similar observation was made by Stewart (1935).

No information is available as to the amount of phosphorus in the shell membrane. Romonoff and Romonoff (1949) reported that the shell membrane contained approximately 10 percent inorganic matter, of which the largest percent was calcium. Stewart (1935) reported that calcium carbonate was absent in the shell membrane. If this is correct, the minute quantities found in the membranes in this study must have been in the form of di- or tricalcium phosphate.

Analyses were carried out on the "inorganic" portion of the shell for the above mentioned elements, and the results are summarized in Table 31. The phosphorus concentrations

Table 30

RELATIVE PHOSPHORUS, CALCIUM, AND PROTEIN CONTENT OF EGG SHELL MEMBRANES[1]

Lot No.	Supplements to Basal Diet (gm./kg.)		Phosphorus (ppm)	Calcium (%)	Protein (%)
	Soft Phos.	Dical. Phos.			
6	45.00	-----	126	0.066	97.31
7	13.75	14.80	128	0.069	96.54
8	27.50	8.30	128	0.068	96.52
9	-----	21.30	122	0.067	97.89
18	-----	-----	121	0.067	97.52

1 Moisture free basis.

Table 31

RELATIVE PHOSPHORUS, CALCIUM, AND PROTEIN CONTENT OF THE SHELL WITHOUT THE MEMBRANE

Lot No.	Supplements to Basal Diet (gm./kg.)		Phosphorus (ppm)	Calcium (%)	Protein (%)
	Soft Phos.	Dical. Phos.			
6	45.00	-----	1039	37.68	2.35
7	13.75	14.80	989	37.09	2.41
8	27.50	8.30	984	37.73	2.44
9	-----	21.30	1005	37.93	2.58
18	-----	-----	961	37.95	2.45

varied considerably between treatments (1039 to 961 ppm). The relative phosphorus level of the shell without the membrane was slightly higher than that obtained with the same shell having the membrane intact (Table 32). The calcium levels were also slightly higher in the shell without the membrane than in the shell with the membrane intact. The protein content of the shell when separated from the membrane (Table 31) ranged from 2.58 to 2.35 percent, whereas for the shell with the membrane intact, the protein content of the shell ranged from 4.60 to 4.74 percent. The protein values are similar to those of Almquist (1934), who stated that the "inorganic" portion contained 2.41 percent protein. It is not known with certainty whether the protein found in the shell is a part of the "inorganic" shell, or just protrusions of the membrane into the shell. Stewart (1935) concluded that the latter was true.

The analysis of shell and membrane separately indicates that the observed changes with regard to phosphorus were apt to appear in the "inorganic" portion of the shell. These analyses will be of value in the future work on egg shells planned at this institution.

Tibia Analysis

Upon termination of the study, ten hens from each lot were sacrificed and their left tibias retained for ashing and chemical analysis. The percent bone ash for each lot is

Table 32

RELATIVE PHOSPHORUS, CALCIUM, AND PROTEIN CONTENT OF SHELL WITH THE MEMBRANE

Lot No.	Supplements to Basal Diet (gm./kg.)		Phosphorus (ppm)	Calcium (%)	Protein (%)
	Soft Phos.	Dical. Phos.			
6	45.00	-----	1017	36.78	4.74
7	13.75	14.80	959	36.40	4.60
8	27.50	8.30	926	36.48	4.65
9	-----	21.30	971	36.46	4.75
18	-----	-----	927	36.50	4.73

summarized in Table 33. The lot receiving all dicalcium phosphate (21.3 gm./kg., Lot 9) had the highest average percent bone ash (64.28%), whereas the lot receiving no supplemental phosphorus had the lowest average percent ash (62.78%, Lot 18). This difference is insignificant, since the ash of the individual bones within each lot varied approximately four percent. However, Gillis et al. (1953) showed a definite change in percent bone ash with the level of phosphorus in the diet of laying hens. These authors got higher percent bone ash when a high level of dicalcium phosphate was fed, than when the diet contained a high level of phytin phosphorus. These authors further showed that the bone ash for the lots receiving 1.33 percent dicalcium phosphate was 64.1 percent. This is approximately the same value that was obtained from the lot receiving all dicalcium phosphate in the study reported herein (Lot 9, Table 33).

The ash of at least three of the tibias from each lot was analyzed for their calcium and phosphorus content. The average percent calcium and phosphorus content for each lot is summarized in Table 33. The calcium and phosphorus contents of the tibias were constant in all lots. Average phosphorus contents of the tibias were 11.04 percent, while that of the calcium was 24.78 percent. This gave a calcium--phosphorus ratio of 2.24. This is a further indication that there was no difference in the percent bone ash between

Table 33

EFFECT OF PHOSPHORUS SUPPLEMENTS ON BONE ASH, CALCIUM, AND PHOSPHORUS CONCENTRATIONS, AND CA:P RATIOS OF TIBIAS FROM S. C. WHITE LEGHORN PULLETS

Lot No.	Supplements to Basal Diet (gm./kg.)		Bone Ash (%)	Phos. (%)	Calcium (%)	Ca:P
	Soft Phos.	Dical. Phos.				
6	45.00	-----	63.23	11.07	24.81	2.24
7	13.75	14.90	63.30	11.24	25.18	2.24
8	27.50	8.30	63.30	10.98	24.60	2.24
9	-----	21.30	64.28	10.94	24.72	2.26
18	-----	-----	62.78	10.99	24.59	2.24

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treatments. Had there been a difference in the percent bone ash, the percent calcium and phosphorus of the tibias would have been altered, thereby altering the Ca:P ratio.

The information concerning the calcium and phosphorus content of laying hens is very limited. A number of percent bone ash measurements have been recorded; however, the relative calcium and phosphorus concentrations were not pursued. It would seem that by determining the concentration of the two major elements present in the bone and determining their ratios, a better record of bone development could be obtained.

It has been shown by O'Rourke et al. (1952) that the chick at ten weeks of age, fed the recommended levels of calcium and phosphorus, had approximately 55 percent bone ash, but again, no information is given as to the relative concentration of calcium and phosphorus.

CONCLUSION

The lot receiving no supplemental phosphorus had significantly ($P < .01$) higher rate of egg production than any of the supplemented lost. Egg production was depressed in all lots receiving supplemental phosphorus when compared with the lot receiving no supplemental phosphorus. There was no significant difference ($P < .05$) among the phosphorus supplemented lots. These results, along with those of previous workers tends to indicate that the laying hen does not require more than 0.41 percent total phosphorus for egg production.

The lot fed no supplemental phosphorus (Lot 18) had a better feed conversion. This was evidenced when expressed on either a lb./doz. eggs or gm./gm. of egg basis. These values for the negative control were 4.29 and 2.69 respectively, while the averages for supplemental lots were 4.85 and 3.03 respectively.

There was no significant difference ($P > .05$) in the percent hatchability of fertile eggs. The mortality which occurred in the study could not be directly associated with the phosphorus treatment fed. There was a greater loss of weight in the negative control than in the other lots tested, however, it is apparent from the results of this study that the maintaining of body weight was not necessary for maximum egg production, since the lot fed no supplemental phosphorus had the highest rate of egg production and also the greatest loss in body weight.

The lots receiving the higher levels of dicalcium phosphate (Lots 7 and 9) had a significantly heavier ($P < .01$) egg than the other lots tested. However, the phosphorus content of the egg yolk was unaltered.

The height of the albumin, the Haugh Index, U. S. D. A. grade distribution, the mottling of the yolks, total blood spots were all used as criteria for measuring the internal quality of the egg. Significant differences were observed between treatments in all of these measurements, excepting the mottling of the yolk. Whenever a significant difference was observed, it was observed in Lot 18, receiving no supplemental phosphorus, and in at least one supplemented lot. This indicates that either the basal diet in the study was not sufficiently low in total phosphorus to measure the effect of supplemental phosphorus on the internal quality of the egg, or the internal quality of the egg is not affected by phosphorus supplements.

Percent shell, absolute weight of the shell, and the shell index were used as criteria for measuring the quality of the shell. The absolute weight of shell and percent shell and shell index declined in all lots as the study progressed. The lot receiving no supplemental phosphorus was found superior in all three of the measurements outlined above. However, one of the supplemented lots was also found to be superior when the absolute weight and percentage shell were considered, indicating again that either the basal diet was not sufficiently low in phosphorus to measure the effects of phosphorus supplements on the external quality of the egg, or possibly, these measurements were not affected by the phosphorus supplement fed.

Analysis was carried out on the egg shell for calcium, protein, and phosphorus. Calcium and protein remained relatively constant throughout the study, but the phosphorus concentration of the shell declined consistently in all lots regardless of dietary treatment, as the study progressed. The average rate of decline for all lots ranged from 126±26 to 954±30 ppm. Regression analysis indicated a significant difference in the rate of decline of shell phosphorus between the supplemented and unsupplemented lots. The rate of decline was curvilinear, being accelerated as the study progressed.

There was a positive correlation between the rate of decline in shell phosphorus concentration and the decline in the average percent shell.

No differences in the percent bone ash between the supplemented lots and the unsupplemented lot were observed. Analysis of the bone for calcium and phosphorus indicated that the percentage composition of these elements remained relatively constnat between treatments.

REFERENCES

Almquist, H. J., and B. R. Burmester, 1934. Characteristic of an abnormal type of egg shell. Poultry Sci. 13:116-122.

Association of Official Agricultural Chemists, 1950. Official and tentative methods of analysis. 7 ed., Washington, D. C.

Baker, R. C., F. W. Hill, A. van Tienhoven, and J. H. Bruckner, 1957. The effect of nicarbazine on egg production. Poultry Sci. 36:718.

Bird, H. R., and C. D. Caskey, Jr., 1943. Amorphous calcium metaphosphate as a phosphorus supplement for chicks. Poultry Sci. 22:333.

Branion, H. D., 1938. Minerals in poultry nutrition. Sci. Agri. 18:217-240.

Bruce, H. M., and R. K. Callow, 1934. Cereals and Rickets. The role of inositol hexaphosphoric acid. Biochem. J. 28:517.

Buckner, G. D., J. H. Martin, and A. M. Peters, 1923. Calcium metabolism in the laying hen. Kentucky Agri. Exp. Sta. Bull. 250.

Carver, J. S., J. R. Evans, and J. McGinnis, 1946. Calcium and phosphorus and Vitamin D interrelationship in the nutrition of the growing chick. Poultry Sci. 25:294-297.

Common, R. H., 1936. Observation on the mineral metabolism of pullets. J. Agri. Sci. 26:85.

Common, R. H., 1939. Observation on the mineral metabolism in poultry. Nature 143:379-380.

Conrad, R. M., 1939. The effect of high temperature on the blood calcium of laying hens. Poultry Sci. 13:327-329.

Duncan, D. B., 1955. The new multiple range and F test. Biometrics 11:1-42.

Evans, R. J., and J. S. Carver, 1942. The calcium and phosphorus requirements of single comb White Leghorn pullets. Poultry Sci. 21:469.

Evans, R. J., and J. S. Carver, 1943. Defluorinated rock phosphate may replace bone meal in laying rations. Washington Agri. Exp. Sta. Circular No. 6.

Evans, R. J., J. S. Carver, and A. W. Brant, 1944a. The influence of dietary factors on egg shell quality. I Phosphorus. Poultry Sci. 23:9-15.

Evans, R. J., J. S. Carver, and A. W. Brant, 1944b. The influence of dietary factors on egg shell quality. II Calcium. Poultry Sci. 23:36-42.

Farnsworth, G. M., and A. W. Nordskoy, 1955. Genetic differences in shell characteristics and other egg quality factors. Poultry Sci. 34:16.

Federal Register, 1955. Regulations governing the grading and inspection of shell eggs and United States standards, grades and weight classes for shell eggs. U.S.D.A. Agricultural Marketing Service Bull. 246.

Froning, G. W., and E. M. Funk, 1958. Seasonal variations in quality of eggs laid by caged layers and their sisters on the floor. Poultry Sci. 37:215.

Gerry, R. W., C. W. Carrick, R. E. Roberts, and S. M. Hauge, 1946. Phosphate supplements of different fluorine content as sources of phosphorus for chickens. Poultry Sci. 25:401.

Gerry, R. W., C. W. Carrick, R. E. Roberts, and S. M. Hauge, 1947. Phosphate supplements of different fluorine content as sources of phosphorus for chickens. Poultry Sci. 26:323.

Gerry, R. W., C. W. Carrick, R. E. Roberts, and S. M. Hauge, 1949. Rock phosphate in laying rations. Poultry Sci. 28:22.

Gillis, M. B., K. W. Keane, and R. A. Collins, 1957. Comparative metabolism of phosphate and inorganic P^{32} by chicks and poults. J. Nutrition 62:13.

Gillis, M. B., L. C. Norris, and G. F. Heuser, 1948. Phosphorus utilization by chicks. J. of Nutrition 35:195.

Gillis, M. B., L. C. Norris, and G. F. Heuser, 1949. The effects of phytin on the phosphorus requirement of the chick. Poultry Sci. 28:283.

Gillis, M. B., L. C. Norris, and G. F. Heuser, 1951. Biological availability of inorganic phosphates. Poultry Sci. 30:914.

Gillis, M. B., L. C. Norris, and G. F. Heuser, 1953. Phosphorus requirements of hens. Poultry Sci. 32:997.

Gillis, M. B., L. C. Norris, and G. F. Heuser, 1954. Studies on the biological value of inorganic phosphates. J. Nutrition 52:115-126.

Grau, C. R., and P. A. Zweigart, 1953. Phosphatic clay as a phosphorus source for chicks. Poultry Sci. 32:500-503.

Haag, J. R., 1939. The calcium and phosphorus contents of chickens of various ages. Poultry Sci. 18:279.

Halpin, J. G., and A. R. Lamb, 1932. The effect of ground phosphate rock fed at various levels on the growth of chicks and on egg production. Poultry Sci. 11:5-13.

Harris, R. S., 1955. Phytic acid and its importance in human nutrition. Nutrition Reviews 13:257.

Harris, R. S., and J. W. M. Bunker, 1935. The phytin phosphorus of the corn component of a rachitigenic diet. J. of Nutrition 9:301.

Hartwood, W. W., 1956. Typical analysis of Dynafos. International Minerals and Chemical Corp.

Haugh, R. R., 1937a. The Haugh Unit for measuring egg quality. The U. S. Egg and Poultry Magazine 43:552.

Haugh, R. R., 1937b. An easy method for finding Haugh Units for interior egg quality. The U. S. Egg and Poultry Magazine 43:760.

Hays, F. A., 1937. Inheritance of egg shape, shell color, and shell texture. Poultry Sci. 16:353.

Hays, F. A., and A. H. Sumbardo, 1927. Physical character of eggs in relation to hatchability. Poultry Sci. 6:196-200.

Hendricks, W. A., A. R. Lee, and A. B. Godfrey, 1931. The effects of cod liver oil and ultra violet irradiation as influenced by oyster shell in the diet of confined laying hens. J. Agri. Research 43:517.

Heuser, G. F., L. C. Norris, J. McGinnis, and M. L. Scott, 1943. Further evidence of the need for supplementing soybean meal chick rations with phosphorus. Poultry Sci. 22:269.

Heywang, B. W., 1956. The effect of supplemental methionine in a corn-soybean diet for laying chickens. Poultry Sci. 35:462.

Hicks, A. F., Jr., 1958. Heritability and correlation analyses of egg weight, egg shape, and egg number, in chickens. Poultry Sci. 37:967.

Huston, T. M., 1958. The influence of high environmental temperatures on egg size, shell quality, and albumin height of domestic fowl. Poultry Sci. 37:1213.

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

Johnson, E. C., R. E. Phillips, and G. A. Donovan, 1953. Utilization of soft phosphate with colloidal clay. Poultry Sci. 32:907.

Jowsey, R. R., M. R. Berlie, W. T. Spinks, and J. B. O'Neil, 1956. Uptake of calcium by the laying hen and subsequent transfer from egg to the chick. Poultry Sci. 35:1234.

Kennard, D. C., 1919. The protein requirement of growing chicks. J. of American Association of Instructors and Investigators in Poultry Husbandry VI: No. 2, 12.

Kennard, D. C., 1921. Rations and methods of feeding chicks to prevent loss when grown in confinement. J. of American Association of Instructors and Investigators in Poultry Husbandry VII: No. 3, 20.

Kilpatrick, L., A. W. Brant, and H. L. Shrader, 1958. Equipment and methods for measuring egg quality. U.S.D.A. Agricultural Marketing Service Bull. 246.

Kirk, P. L., 1950. Kjeldahl method for total nitrogen. Anal. Chem. 22:354.

Koenig, R. A., and C. R. Johnson, 1942. Colorimetric determination of phosphorus in plant material by the molybdivanado-phosphoric acid method. Ind. Eng. Chem., Anal. Ed. 14:155.

Lorenz, F. W., I. Perlman, and I. L. Charkoff, 1938. Phosphorus deposition in the egg as measured with radioactive phosphorus. Am. J. of Physio. 138:318-327.

Lowe, J. T., H. Steenbock, and C. H. Krieger, 1939. Cereals and rickets. IX The availability of Phytin-P to the chick. Poultry Sci. 18:40-44.

Lyons, M., 1939. Some effects of manganese on egg shell quality. Kentucky Agri. Exp. Sta. Bull. 374.

Mac Intyre, T. M., and M. H. Jenkins, 1955. Effect of different feeding methods on the efficiency of egg production. Poultry Sci. 34:376.

McGinnis, J., L. C. Norris, and G. F. Heuser, 1944. Poor utilization of phosphorus in cereals and legumes by chicks for bone development. Poultry Sci. 23:157-159.

Miller, M. W., and G. E. Bearse, 1934. Phosphorus requirement of laying hens. Washington Agri. Exp. Sta. Bull. 306.

Miller, M. W., V. V. Joukovsky, 1953. Availability of phosphorus from various phosphate materials for chicks. Poultry Sci. 32:78-81.

Mitchell, H. H., and H. J. McClure, 1937. Mineral nutrition of farm animals. Nat. Research Council Bull. 99.

Motzok, I., D. A. Branion, and H. D. Branion, 1956. Utilizationoof phosphorus from various phosphate supplements by chicks. Poultry Sci. 35:627-649.

Motzok, I., D. A. Branion, and H. D. Branion, 1957. Availability of phosphorus supplements to chicks. Poultry Sci. 36:1089-1091.

National Research Council, 1937. Recommended Nutrient allowances for domestic animals. No. 1, Recommended nutrients allowances for poultry.

Nikolaiczuk, N. R., R. M. Bethke, and T. S. Sutton, 1949. Retention by the chick of phosphorus from organic and inorganic sources. Poultry Sci. 28:777.

Norris, L. C., G. F. Heuser, A. T. Ringrose, and H. S. Wilgus, Jr., 1934. Studies of calcium requirement of laying hens. Poultry Sci. 13:308-309.

O'Rourke, W. F., H. R. Bird, P. H. Phillips, and W. W. Craven, 1954. The effect of low phosphorus rations on egg production and hatchability. Poultry Sci. 33:1117-1122.

O'Rourke, W. F., P. H. Phillips, and W. W. Craven, 1952. The phosphorus requirements of growing chickens as related to age. Poultry Sci. 31:962.

O'Rourke, W. F., P. H. Phillips, and W. W. Craven, 1955. The phosphorus requirement of growing chickens and laying pullets fed practical rations. Poultry Sci. 34:47-55.

Pepper, W. F., S. J. Slinger, and J. D. Summers, 1958. On the phosphorus requirement of laying hens. Poultry Sci. 37:1233.

Phillips, A. G., and S. M. Hauge, 1925. Soybean oil meal in rations for laying pullets. Purdue Agri. Exp. Sta. Bull. 293.

Piper, C. S., 1950. Soils and Plant Analysis. Interscience Publishers, Inc., New York, N. Y.

Polin, D., W. H. Ott, and O. H. Siegmund, 1957. The incidence and degree of yolk mottling in eggs from hens fed diets with and without nicarbazin. Poultry Sci. 36:524.

Reynolds, D. S., W. L. Hill, and E. D. Jacob, 1944. Dilute hydrochloric acid as a solvent for phosphates with special reference to defluorinated phosphates and other materials used as phosphorus supplements for livestock. J. Assoc. Off. Agri. Chem. 27:559-571.

Romanoff, A. L., and A. J. Romanoff, 1949. The Avian Egg. John Wiley and Sons, Inc., New York, N.Y.

Rosenberg, M. M., and T. Tanaka, 1951. Effect of Temperature on egg weight in Hawaii. Poultry Sci. 30:745.

Sanchelli, V., 1951. Phosphates in Agriculture. The Davison Chemical Company.

Schaible, P. J., 1941. Minerals in poultry nutrition -- a review. Poultry Sci. 20:278-388.

Sherwood, R. M., 1932. Lime and phosphoric acid requirements for chicks. Texas Agri. Exp. Sta. Bull. 462.

Singsen, E. P., L. D. Matterson, and A. Kozeff, 1950. Phosphorus in poultry nutrition IV. Radioactive phosphorus as a tracer in studying the metabolism of phytin by the turkey poults. Poultry Sci. 29:635.

Singsen, E. P., and H. H. Mitchell, 1944. Soybean meal chick rations need no inorganic phosphorus supplements. Poultry Sci. 23:152-153.

Singsen, E. P., and H. H. Mitchell, 1945. Phosphorus in poultry nutrition I. Relationship between phytin and different sources of Vitamin D. Poultry Sci. 24:479-480.

Singsen, E. P., and H. M. Scott, 1946. Phosphorus in poultry nutrition II. Sodium acid phosphate, tricalcium phosphate, and bone meal, as sources of phosphorus for growing chicks. Poultry Sci. 25:302.

Smith, A. H., C. M. Winget, and J. R. Blackard, 1954. Transfer of radio phosphorus to the hen's egg under controlled environment, as traced with radio phosphorus. Poultry Sci. 33:908.

Spitzer, R. R., G. Maruiyama, L. Michaud, and P. H. Phillips, 1948. The role of Vitamin D in the utilization of phytin phosphorus. J. Nutrition 35:185.

Spitzer, R. R., P. H. Phillips, 1945. Enzymatic relationships in the utilization of soybean oil meal phosphorus by the rat. J. Nutrition. 30:183-192.

Stewart, G. F., 1935. The structure of the hen's egg shell. Poultry Sci. 14:24.

Taylor, L. W., and I. M. Lerner, 1939. Inheritance of egg shell thickness in leghorn pullets. J. of Agri. Research 58:383-396.

Taylor, L. W., and O. H. Martin, 1928. Factors influencing thickness of egg shell. Poultry Sci. 8:39-44.

Taylor, T. G., and J. H. Moore, 1956. Skeletal depletion in hens laying on a low calcium diet. Brit. J. Nutrition 10:250.

Titus, H. W., 1955. The Scientific Feeding of Chicks. The Interstate Printers and Publishers, Inc.

Warren, D. C., and R. L. Schnepel, 1940. The effect of air temperature on egg shell thickness in the fowl. Poultry Sci. 19:67-72.

Wheeler, W. P., 1903. The importance of mineral matter and the value of grit for chickens. New York Agri. Exp. Sta. Bull. 242.

Wilgus, H. S., Jr., 1931. The quantative requirement of the growing chick for calcium and phosphorus. Poultry Sci. 10:107.

Wilgus, H. S., Jr., and A. Van Wagenen, 1936. The height of the firm albumin as a measure of its condition. Poultry Sci. 15:319.

Wilhelm, L. A., 1940. Some factors affecting variation in eggshell quality. Poultry Sci. 19:246-253.