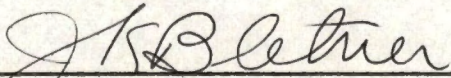


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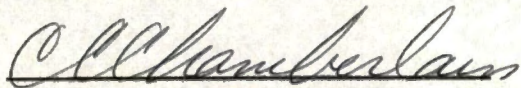
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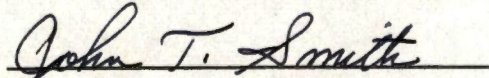
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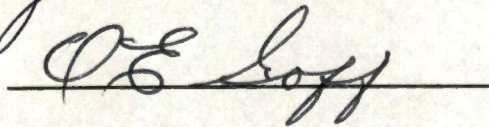
I am submitting herewith a dissertation written by Ram Dhari Singh entitled "The Effect of Ca and P Levels in Laying Diets on the Bone Density Index, Bone Breaking Strength, Egg Specific Gravity, Egg Production, and the Incidence of Cage Layer Fatigue." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.


Major Professor

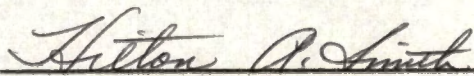
We have read this dissertation
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Accepted for the Council:


Vice Chancellor for
Graduate Studies and Research

THE EFFECT OF CA AND P LEVELS IN LAYING DIETS ON THE BONE DENSITY INDEX,
BONE BREAKING STRENGTH, EGG SPECIFIC GRAVITY, EGG PRODUCTION,
AND THE INCIDENCE OF CAGE LAYER FATIGUE

A Dissertation
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
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by
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ABSTRACT

A total of four trials involving 990 S. C. White Leghorn-type pullets were conducted to study the response of caged layers to various Ca and P levels as measured by bone density index, bone breaking strength, egg production, and egg specific gravity. An X-ray type bone densitometer was used to determine bone density index and an Allo-Kramer shear press to determine the breaking strength of tibia.

Whenever layers were fed a diet containing 1.5 percent Ca, they maintained about 50 percent egg production. Specific gravity score of their eggs and the bone density of tibia were found significantly lower than those of birds fed diets with the higher levels of Ca.

When layers were transferred to lower levels of Ca and P, egg shell quality and bone density indexes were reduced quickly. Egg production was reduced but the rate of this reduction may have been affected by the level of Ca and P in the diet prior to the feeding of the low Ca and P diets.

Transferring layers from diets containing 2.25 or 2.75 percent Ca and 0.6 percent P to diets containing 3.0 percent Ca and 0.8 percent P had no marked effects on egg production, egg specific gravity, bone density index, or bone breaking strength.

It appears that the dietary Ca requirement for egg production is less than 3.0 percent, that for high egg specific gravity it will be no more than 3.50 percent, and that for high bone density index no more than 3.0 percent. The dietary P requirement for egg production

appears to be no more than 0.6 percent; that for high egg specific gravity score, 0.4 percent; and for high bone density, 0.8 percent of the diet.

Birds with symptoms of leg weakness (cage layer osteoporosis) were found in lots fed all diets. The highest incidence of this condition was in those birds fed the 1.5 percent Ca diet.

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I. INTRODUCTION

In present day commercial egg production pullets are usually placed in cages in contrast to the previous practice of keeping them on solid floors. This practice has apparently increased the need for more phosphorus (P), in addition to other nutrients, because a recycling of feed is not possible for birds kept in cages, whereas chickens kept on floors may supplement their feed by the consumption of their excreta.

A considerable amount of work has been reported concerning the requirements of calcium (Ca) and P for laying hens, but no definite satisfactory levels have yet been determined which will alleviate the incidence of a kind of leg weakness, first reported by Couch (1955) as "Cage Layer Fatigue."

In cage layer fatigue, the walls of the bones become very thin, hard, brittle and easily broken, rather than soft or rubbery as they often are in rickets. At processing plants, caged birds are often downgraded because of bone shattering. Floor-raised birds are generally preferred to caged birds. This situation has created an economic problem for the poultry industry. Therefore, experiments were designed to study the response of caged layers to various Ca and P levels as measured by bone density index, bone breaking strength, egg production, and specific gravity when:

1. Ca levels are varied and Ca:P ratios maintained at 3.75:1.

2. Graded levels of P are included in diets containing 3 and 3.5 percent Ca.
3. Layers are fed a 3.0 percent Ca-0.7 percent P diet and changed to a lower level of Ca and P at different ages.
4. Layers are fed different levels of Ca and P for eight weeks and changed to higher and lower levels of Ca and P for eight weeks.

II. REVIEW OF LITERATURE

Cage Layer Fatigue

Cage layer fatigue is a disorder of the skeleton of layers maintained in cages characterized by a decrease in the amount of the hard tissue without any change in the volume or configuration of the bones. McClean and Urist (1968) have defined cage layer fatigue as a form of osteoporosis in some respects resembling the disorder in man, and indicated that it is found most often in the White Leghorn hen bred for heavy egg production. Scott, Nesheim and Young (1969) have concluded that cage layer fatigue is a type of osteoporosis.

Cage layer fatigue was first described by Couch (1955) as a form of leg weakness in caged birds that disappeared when the affected birds were removed from cages. Grumbles (1959) indicated that in 1955 he recognized cage layer fatigue as a significant disease problem in Texas. Couch (1959) concluded that cage layer fatigue was caused by a nutritional deficiency and that generally this occurred in pullets laying at a very high rate. Riddell et al. (1968) observed that birds affected with cage layer fatigue had bones which were easily broken, and had deformation of sternum and ribs.

Breed and Genetic Difference

Breed and genetic differences have also been given attention in consideration of cage layer fatigue. Francis (1957) observed cage layer fatigue in different breeds of chickens. He found that the incidence of cage layer fatigue, as observed in individual flocks, may vary according to the type of chickens. Recovery by affected birds may also be influenced by the breed and early removal from the cages. The incidence of cage layer fatigue in breeds studied varied from 0.65 to 3.95 percent. Bell and Siller (1962) made a study of the pathological symptoms of 40 cases of cage layer fatigue among heavy-laying Brown Leghorn pullets. The study revealed that a genetic susceptibility apparently existed. Ewing (1963) concluded that the incidence of cage layer fatigue, as observed in individual flocks, may vary according to the breed of the chicken.

Calcium

Simpson et al. (1964) fed a low level of calcium (2 percent) to pullets and found no symptom of leg weakness which they identified as cage layer fatigue. Salman, Ali and McGinnis (1969) reported no cage layer fatigue in experiments when layers were fed diets of either 2.75 percent or 4.5 percent calcium and 0.3 percent plant phosphorus, and the mortality in these experiments was considered within the normal range. Henrikson et al. (1970) induced osteoporosis by feeding a low level of Ca (low = 0.12 percent; adequate = 0.54 percent) and high phosphorus (high = 1.20 percent; adequate = 0.42 percent) for 41 weeks

to adult beagles. Krook et al. (1971) studied the reversibility of nutritional osteoporosis in adult beagles, and suggested that increasing the dietary calcium, if instituted before structural damage has occurred, may reverse osteoporosis by increasing bone ash and bone density.

Scott and Mullenhoff (1970) recommended that two-thirds of the Ca supplement be in the form of oyster shell. They determined the Ca concentration in blood at different hours. They found that at 4:00 a.m. the hens receiving two-thirds oyster shell and one-third ground limestone had 17 percent higher blood calcium levels than those receiving the same total Ca level supplied entirely as limestone in powder form. They suggested that oyster shell dissolved more slowly than the powder forms of Ca sources in the gut so generally at night when the egg shell is being formed Ca is available for shell formation from the oyster shell that is "metering" from the gizzard into the gut. It is quite possible that when the dietary Ca is available for shell formation over a longer period of time that the layers will not remove as much Ca from the bones. Therefore, the incidence of cage layer fatigue may be reduced.

Phosphorus

Simpson et al. (1964) fed low phosphorus diets (0.34 percent) to pullets. These developed leg weakness and had definite histological evidence of bone damage. Harms (1968) reported that chickens getting a marginal or low level of phosphorus were more prone to the cage layer

fatigue syndrome. He speculated that a marginal phosphorus deficiency might be implicated in this disease. Singsen et al. (1969) reported that replacing dietary dicalcium phosphate with Curacao phosphate resulted in an increased mortality due to an increase in the incidence of cage layer osteoporosis.

Fluorine

The use of fluoride as a treatment for osteoporosis has been practiced primarily in medical science. Purves (1962) reported that fluorine changed a negative calcium balance to a positive balance, or made positive balance to more positive, thus relieving the disease. Rich, Ensink and Ivanovich (1964) reported that either increased gastrointestinal absorption, or reduced excretion of endogenous calcium, or both, caused decreased fecal calcium. Cass et al. (1966) studied fluorine incorporation in bone. They found that after fluorine treatment a decrease in the fecal and urinary excretion of calcium caused a higher retention of calcium in the body. Korn (1969) studied the effect of two levels of fluorine in drinking water of humans, 0.05 (normal water) and 1.0-1.2 parts per million fluorine. In observations throughout 22 years, he found no significant differences that could be attributed to the effects of different levels of fluoride on osteoporosis. Henrikson et al. (1970) induced osteoporosis in adult beagle dogs and the effect of fluoride to modify this condition was examined by giving different levels of fluoride to the diet. Radiographic and microradiographic examination of bones revealed no effect on the degree of

osteoporosis attributable to the added fluorine. Singsen et al. (1969) fed sodium fluoroide at the levels 12, 24, and 48 mg./Kg. of feed to chickens and found that fluorine had no effect on the incidence of cage layer fatigue.

Egg Shell Quality

The egg shell has considerable effect on the economic value of an egg. It should be sufficiently strong to resist breakage in transportation.

Dietary Ca and P

The level of dietary calcium has a very great influence on egg shell quality. Wolford and Tanaka (1970) made a very exhaustive review of factors influencing egg shell quality. They concluded that 18 authors agreed that less than 2 percent Ca in the diet significantly decreased egg shell quality in chickens. Twenty-one authors stated that an excess of 3 percent Ca and nine authors an excess of 4 percent Ca in diet, increased egg specific gravity in chickens.

Mitchell and McClure (1937) recommended a daily intake of 1.60 grams per hen or 3.52 percent dietary level based upon 50 percent utilization of Ca as net requirement for 75 percent production. In the years 1954 and 1960 a level of 2.25 percent of calcium for layers was recommended, while in 1966 the National Research Council raised its previous recommendations to 2.75 percent. This latter recommendation was retained in their 1971 report. This increase in the recommended level of

dietary calcium undoubtedly was due to the large number of studies prior to 1966 which indicated that 2.25 percent calcium was too low for high production hens.

Miller and Bearse (1934) found that egg shell quality was not influenced by the levels of 2.22 and 3.0 percent of Ca. Norris et al. (1934) reported that 1.8 percent Ca was optimum for egg shell strength. Gutowska and Parkhurst (1942) found no significant difference in egg shell breaking strength when they fed three levels of Ca, 2.35, 2.77; and 3.95 percent. Evans, Carver, and Brant (1944) found that 3.0 percent Ca and 0.8 percent P in the diet gave more satisfactory results than those containing 2.25 percent Ca and 0.8 percent P when egg shell thickness was used as the criterion. At the one percent level of calcium, egg shell quality was reduced. Petersen et al. (1960) concluded that the level of 2.25 percent Ca was not adequate for production of eggs with best shell qualities. Hunt and Aitken (1962) reported that specific gravity of eggs was significantly depressed by low dietary calcium (1.75 percent). Hinnners, Gholson and Richardson (1963) found that shell thickness increased as the dietary Ca levels increased up to 5.5 percent. Gerry and Bird (1967) have shown that egg shell thickness did not increase with a high level (3.75 percent) of calcium in the diet, however, bone ash was increased slightly.

Jenkins and Tyler (1960) found that dietary phosphorus levels of 0.752 gram per day to 1.504 grams per day failed to affect shell thickness and neither was there any interaction between Ca and P. Walter and Aitken (1962) fed a level of 0.4 to 0.7 percent P to layers

in cages and reported that 0.4 percent was adequate for a desirable specific gravity score of eggs. Hanners, Gholson and Richardson (1963) also reported that increasing P levels from 0.705 to 1.018 percent failed to show any significant change in shell thickness. Hunt and Chancey (1970) found that the specific gravity of eggs was highest for eggs from hens kept on the floor fed the 0.36 percent P diet and decreased as the level of P in the diet increased, but a reduction in the total dietary P from 0.72 to 0.39 percent had no effect on the egg shell quality of caged birds.

Environment

Environmental temperature also has an influence on egg shell quality. As early as 1933 attention was given by Conrad to determine the effect of temperature on blood calcium level as well as on egg shell quality. He found a 25 to 30 percent decrease in blood calcium level when the environmental temperature rose from 70 degrees to 90 degrees F. He interpreted his result to indicate that the reduction in thickness of egg shells can probably be attributed directly to the decreased calcium carrying capacity of the blood. Warren and Schnepel (1940) subjected hens and pullets to 90 degrees F. They showed a striking reduction in shell quality. These workers also suggested that high humidity accentuates the depressing effect of high temperature on shell thickness. Petersen et al. (1958) demonstrated the influence of environmental temperatures above 70 degrees F. in decreasing shell quality as determined by specific gravity. They also observed a continued decline in specific gravity which they reported to be due to increasing

age combined with the high temperature conditions. Frøning and Funk (1958) reported that in general as high and low temperature trends are followed, greater shell thickness is associated rather closely with cooler temperature and lower shell thickness with periods of high temperatures. Tyler and Greake (1958) found that there was a significant difference in egg shell thickness between the June period and the period of January, February to March and April favoring a thicker shell in the latter period. Petersen et al. (1960) reported that with an environmental temperature of less than 70 degrees F., 3.75 percent Ca is not necessary for good shell quality. With a temperature of 70 degrees F. or greater a calcium level of 4.5 to 5.0 percent was suggested. Durham et al. (1960) observed that the specific gravity of eggs declines at the onset of warm weather. This decline was smaller in birds fed higher calcium diets. Mueller (1961) reported that a constant temperature of 90 degrees F. depressed egg shell quality as compared with a constant temperature of 55 degrees F. Wolford and Tanaka (1970) concluded that an environmental temperature above 32 degrees C. resulted in lower egg shell quality.

Egg Production

A certain level of Ca and P is essential for optimum egg production. Miller and Bearse (1934) found that a level of Ca between 2.22 and 3.0 percent was suitable for good egg production. Norris et al. (1934) reported that 1.65 percent of Ca was just adequate for egg production. Gutowska and Parkhurst (1942) reported that 3.95 percent of Ca in diet

decreased egg production as compared with the levels of 2.77 and 2.35 percent of Ca in the diet. Sanford and Alder (1969) found that a level of 3.0 percent Ca was most suitable for maximum egg production. Davidson and Boyne (1970) found that if a lower than the required level of Ca is fed in the diet to layers, the lower production was associated with reduced feed intake.

Titus et al. (1937) concluded that the levels of dietary P, 0.9 and 1.2 percent, had no significant effect on egg production. Singsen et al. (1962) reported that higher egg production was obtained with more than 0.4 percent P, but not more than 0.6 percent P. Egg production was depressed with 0.7 percent P in diet. They found no significant difference between 0.4, 0.5, or 0.6 percent level of P in the diet. Harms (1968) fed 0.34 percent total P in the diet which caused a decrease egg production and extremely high mortality. Sanford and Alder (1969) reported that a level of 0.7 percent of P was required for better egg production. Salman, Ali and McGinnis (1969) found that 0.3 percent of plant P in the diet was adequate for egg production with Ca levels of either 2.75 or 4.50 percent of the feed.

Bone Breaking Strength

One of the criteria used in measuring bone mineral is breaking strength. Rowland et al. (1967b) used a modified Allo-Kramer shear press to determine the breaking strength of bones in chicks. They found that the correlation coefficient for tibia ash and tibia breaking strength was +.63 when calculations were made on an individual

bird basis. However, a correlation coefficient of +.98 was found when the average tibia ash was correlated with the average breaking strength. Rowland et al. (1967a) found that egg production type hens, fed a low level of Ca (1.5 percent of diet) for two weeks, had tibiae of lower ash and breaking strength than control hens. The breaking strength (pounds) and bone ash (percent), respectively, were as follows for the different treatment groups: 1.5 percent Ca, 28.9, 59.3; control (3 percent Ca), 29.2, 60.4; 6.0 percent Ca, 37.2, 62.1. Fry (1969) reported similar findings. According to these studies it was suggested that breaking strength is as good a measure of dietary Ca and P levels as is the bone ash. Rowland et al. (1968a) found that the addition of high levels of dietary Ca and the cessation of egg production, through feeding high levels of iodine or dienestrol diacetate, produced higher breaking strength and ash than that of the regular laying birds. Again, Rowland et al. (1968b) observed that the breaking strength of bones from hens maintained on litter was significantly greater ($P < .005$) than from those of birds maintained in cages. Increasing the phosphorus level of the diet from 0.8 to 1.20 percent failed to significantly affect the breaking strength. Rowland and Harms (1970) measured the breaking strength of bones of chickens kept on litter, wire, and in cages, and found breaking a strength of 46.20, 47.20, and 38.10 pounds, respectively, indicating that caged birds have weaker bones.

Bone Density

Different techniques have been developed to determine the bone density or bone mass. It gives the relative mass of the bone with respect to the wedge used. To determine bone mineralization in vivo attention was given as early as 1935 by Sanders as cited by Mack et al. (1939):

For this purpose, Sanders, in 1935, began to use a photometric apparatus in which a ray of light was directed through a certain area of a roentgenogram onto a photronic cell in circuit with a voltmeter; the deflection of the voltmeter was compared with that obtained when the light was passed through each of a series of steps of a density ladder on the same roentgenogram.

Stein (1937) used a photronic apparatus to determine bone mineral. He used a density ladder made of ivory for comparative purpose. Mack et al. (1939) used a microphotometer to make tracings from one fixed landmark to another on a roentgenogram. They made tracings of roentgenograms of both bones and teeth. They also used a density ladder for purposes of comparison. Babcock et al. (1965) measured bone mass using a mono-energetic beam from ^{125}I by measuring its absorption by the tibia. They showed that the above technique would distinguish bone mass changes reflected by a change in total body Ca of the order of 10 percent. Montilla, Babcock and Sunde (1965) observed an increase in bone mineral mass as production diminished late in the laying year. Meyer, Babcock and Sunde (1968) determined bone mineral content by measuring the transmission of a monoenergetic photon beam. The radioactive source which they used was ^{125}I . They claimed the error was

within 3.2 percent. They found a correlation between bone mass units and ash weight per centimeter bone of 0.955 for a live chicken with no flesh equivalent present, and 0.934 when a flesh equivalent material was present.

A bone densitometer designed by Williams was described by Mason and Ruthven (1965). They stated that greater precision is attained by elimination of X-ray film with its error, and by the reduction of scatter through collimation. This eliminates not only the X-ray film from the procedure but approximate linearity is achieved for the absorption curves of the reference standard and test object. The absorption curve from the chosen object is traced on graph paper by an X-ray recorder. The stand travels through the X-ray beam at the rate of one inch (2.5 cm.) per 56 seconds. With a phantom finger, results of the bone densitometer were reproducible within 3 percent. Repeated measurements on human subjects were reproducible within 6 percent, as compared with 12 to 14 percent when film is used.

III. EXPERIMENTAL PROCEDURE

General

Hatching eggs of Babcock B-300 Single Comb White Leghorn-type chickens were obtained from a local hatchery. The eggs were incubated at the Poultry Unit of The University of Tennessee Agricultural Experiment Station, Knoxville. When the chicks were one day old, they were sexed, vaccinated, and placed in houses for floor brooding. For about the first 10 weeks in the brooder house, infra-red heat lamps served as the heat source. At 10 weeks of age, the pullets were moved to a growing shelter. The pullets in all trials were fed a similar starter diet from one day old to 10 weeks of age, and then a growing diet until they were transferred to the laying house (Table I). In the laying house the pullets of trials 1 and 2 were fed a layer diet containing 3 percent calcium and 0.7 percent phosphorus until they were fed the experimental diets (Table I).

In trials 1 and 2 the birds were vaccinated for Newcastle disease and infectious bronchitis at one day, 10 weeks, and 22 weeks of age, and for the fowl pox, at 22 weeks of age. In trials 3 and 4 the pullets were vaccinated for Newcastle disease and infectious bronchitis at one day, 10 weeks, and 21 weeks of age, and for the fowl pox at one day and 21 weeks of age.

Table I

Composition and Calculated Analysis of the Starter,
Grower, and Layer Diets

Ingredients	Starter diet	Grower diet	Layer diet
Yellow corn	63.800	71.875	66.975
Alfalfa meal, 17 percent	2.500	5.000	5.000
Fish meal	2.500 ¹	2.500 ¹	2.500 ²
Vitamin mix	0.600 ¹	0.600 ¹	0.500 ²
Defluorinated rock phosphate	1.500	1.500	1.500
Ground limestone	0.600	1.000	6.000
Salt	0.480	0.500	0.500
Manganese sulfate, 75 percent	0.020	0.025	0.025
Soybean oil meal, 50 percent	25.500	14.500	17.000
Coccidiostat premix	2.500	2.500	-----
Total	100.000	100.000	100.000
Calculated analyses:			
Crude protein (percent)	22.040	17.680	17.190
Productive energy (C/Kg)	2108.000	2128.000	2006.000
Calcium (percent)	0.894	1.057	2.957
Phosphorus (percent)	0.711	0.636	0.621
Available phosphorus (percent)	0.414	0.4217	0.417

¹Vitamin mix supplied the following amounts per kilogram of diet:
4167 I.U. of vitamin A; 749 I.C.U. of vitamin D₃; 4.74 mg. of riboflavin;
11.0 mcg. of vitamin B₁₂; 406 mg of choline; 40.30 mg. of niacin; 7.00
mg. of pantothenic acid and 11.9 mg. of aureomycin.

²Vitamin mix supplied the following amounts per kilogram of diet:
2463 I.U. of vitamin A; 2956 I.C.U. of vitamin D₃; 4.43 mg. of riboflavin;
650 mcg. of vitamin B₁₂; 440 mg of choline; 24.2 mg. of niacin and 4.76
mg. of pantothenic acid.

In all trials, pullets were housed individually in cages having a floor size of 8 x 16 inches. Each diet was replicated and each replicate consisted of 15 birds. Trials 1 and 2 were conducted concurrently as were trials 3 and 4. The pullets in trials 1 and 2 were placed in the laying house in early June while in trials 3 and 4, they were placed in the laying house in the third week of January.

The nonvariables in the experimental diets (Table II) included all feedstuffs used except defluorinated rock phosphate, limestone, and sand. The nonvariables made up 85 percent of each diet. The desired levels of Ca and P were obtained by varying the defluorinated rock phosphate and limestone. Enough sand was added to bring the total for the variable portion to 15 percent of the diet.

Each experimental diet was calculated to contain 17.23 percent crude protein and 1989 Calories of productive energy per kilogram.

The amount of the variable ingredients used in the experimental diets is given in Table III. The first three diets listed for trial 1 were used also in trials 3 and 4.

In all trials a bone densitometer designed by Williams and Mason (1962) was used to determine bone density index. The bone density value is expressed as X-ray equivalent grams of alloy (wedge having 92.8 percent aluminum and 7.2 percent of zinc) per cubic centimeter of bone. The densitometer used a low intensity X-ray beam as a source of energy and utilizes a scintillation detector conjoined with a photomultiplier tube. It incorporates an electronic circuit which utilizes the logarithmic characteristic of the photomultiplier tube to produce

Table II
Nonvariables and Variables Used in Experimental Diets

	Percent
<u>Nonvariables</u>	
Yellow corn	52.00
Fish meal, 65 percent	2.50
Alfalfa meal, 17 percent	5.00
Soybean oil meal, 50 percent	19.76
Vitamin mix ¹	0.50
Salt	0.50
Manganese sulfate, 75 percent	0.03
Animal fat	4.71
Total	85.00
<u>Variables</u>	
	<u>Range</u>
Defluorinated rock phosphate	0.303- 4.630
Limestone	3.090- 8.350
Sand	2.020-11.607
Total	15.00

¹Vitamin mix supplied the following amounts per kilogram of diet: 2463 I.U. of vitamin A; 2956 I.C.U. of vitamin D₃; 4.43 mg of riboflavin; 6.50 mcg. of vitamin B₁₂; 440 mg. of choline; 24.2 mg of niacin; and 4.76 mg. of pantothenic acid.

Table III
Variables in Diets Fed in Trials 1 and 2

Dietary Ca and P		Ca:P ratio	Variables		
Ca	P		Ground limestone	Defluorinated rock phosphate	sand
-----percent-----			-----percent of diet-----		
<u>Trial 1</u>					
1.50	0.4	3.75:1	3.090	0.303	11.607
2.25	0.6	3.75:1	4.415	1.384	9.201
3.00	0.8	3.75:1	5.728	2.465	6.807
3.75	1.0	3.75:1	7.041	3.546	4.413
4.50	1.2	3.75:1	8.350	4.630	2.020
<u>Trial 2</u>					
3.00	0.4	7.50:1	6.946	0.303	7.751
	0.6	5.00:1	6.338	1.384	7.278
	0.8	3.75:1	5.728	2.465	6.807
	1.0	3.00:1	5.118	3.546	6.336
	1.2	2.50:1	4.510	4.627	5.863
3.50	0.4	8.75:1	8.228	0.303	6.469
	0.6	5.83:1	7.621	1.384	5.995
	0.8	4.38:1	7.010	2.465	5.525
	1.0	3.50:1	6.400	3.546	5.054
	1.2	2.92:1	5.792	4.627	4.581

linear absorption curves directly on graph paper by an X-ray recorder (Mason and Ruthven, 1965).

The birds were laid in a cradle with the left leg stretched backward resting on a platform. A light rope with one end attached to the machine and the other to a weight was used to place across the hock joint in an attempt to keep the leg on the platform. This device for restraining the bird discouraged her from moving. The site of X-ray was about 3 cm. from the hock joint on the tibia. The platform on which the leg rested moved vertically 2.56 cm., thus raising the leg. This elevation of the leg caused it to pass through the collimated X-ray beam. This interruption in the intensity of the beam was reflected on the x-y plotter linearly. The wedge was X-rayed after each X-ray of the leg. In trials 1 and 2 the pullets were X-rayed with the feathers on the leg, but in trials 3 and 4 the feathers were removed from the site of X-ray. The area under the curve of graph was calculated by using a compensating planimeter calibrated in square centimeters. The bone density indexes were determined by the methods outlined by Williams and Mason (1962).

Observations were made on those birds which were unable to stand or which showed leg weakness. Sick birds were provided with feed in a special cup placed in their cages. Whenever a bird was unable to stand, she was X-rayed; after recovery, she was X-rayed again.

Egg production records were maintained on each hen. These data were collected for five days per week and then were adjusted for the whole period.

The weight of individual eggs, in grams, laid on the last day of the period was recorded for each lot, and the same eggs were used to determine the specific gravity. The specific gravity of the eggs was determined as a measure of shell quality at the end of each period. Ten salt solutions, ranging in specific gravity from 1.064 to 1.100 by an increment of 0.004, were employed (Thirteenth Tennessee Random Sample Test, 1970).

The birds which had been regularly X-rayed were sacrificed at the end of each experiment. The tibiae of left leg of each bird were cleaned of flesh and broken by an Allo-Kramer shear press (Model No. S-Z HE) to determine the breaking strength of the bones. A guillotine with an inverted "V" shaped notch in the contact edge was attached to a 250-pound ring. The guillotine was lowered at a velocity of 1 cm. per 2.4 seconds against the mid-point of the tibiae. The shear press was modified by providing two blocks on which the bones were laid.

Trial 1

Trial 1 was designed to study the effects of graded levels of P in laying diets when the Ca:P ratios were maintained at 3.75 to 1. The diets were calculated to contain 1.50, 2.25, 3.00, 3.75, and 4.50 percent Ca and 0.4, 0.6, 0.8, 1.0, and 1.2 percent P, respectively. A total of 150 pullets assigned to 10 lots of 15 pullets each were used. Five pullets from each of five lots were X-rayed at the age of 22 weeks. This was at the time the pullets were changed from a grower to a layer diet and four weeks before they were fed their experimental diets.

X-rays were made of five birds per lot when the birds were 26 weeks old and at the end of each 28-day period thereafter for five periods except at the end of period 1 when the bone densitometer was inoperative.

Trial 2

Trial 2 was designed to study the effects of two levels of Ca and graded levels of P as mentioned in trial 1. The diets were calculated to contain 3.00 and 3.50 percent of Ca and 0.4, 0.6, 0.8, 1.0, and 1.2 percent of P. A total of 300 pullets were assigned to 20 lots of 15 pullets each. Five pullets from each of 10 lots were X-rayed at the age of 22 weeks, when the pullets were changed from a grower to a layer diet and four weeks before they were fed the above experimental diets.

X-rays were made of five birds per lot when the birds were 26 weeks old and at the end of each 28-day period thereafter for five periods, except at the end of period 1 when the bone densitometer was inoperative.

Trial 3

Trial 3 was designed to study the effects of length of time laying pullets were fed a 3 percent Ca, 0.7 percent of P diet on their response to diets containing 1.5 percent Ca and 0.4 percent P, 2.25 percent Ca and 0.6 percent P, and 3.0 percent Ca and 0.8 percent P. A total of 270 pullets were assigned to 18 lots of 15 pullets each lot.

Each diet was fed to two replicate lots of pullets throughout the 16 week experimental period. Six other lots were fed the laying diet used at the Poultry Unit (calculated to contain 3.0 percent Ca and 0.7 percent P) for four weeks. Then they were changed to the above experimental diets with each diet being assigned to two replicate lots. The last six lots were fed the laying diet for eight weeks, then they were fed the above experimental diets for eight weeks.

Five pullets from each lot were X-rayed before they were fed the experimental diets and then at the end of each experimental period.

Trial 4

Trial 4 was designed to study the effects of Ca level in the diet of laying hens on their response to higher and lower Ca levels in diets fed after the pullets were 29 weeks of age. A total of 270 pullets were assigned to 18 lots of 15 pullets each lot. The pullets of six replicate lots were fed the laying diet, containing 3.0 percent Ca and 0.7 percent P used at the Poultry Unit, for eight weeks. Another six replicate lots were fed a diet containing 2.75 percent of Ca and 0.6 percent P for eight weeks and the last six replicate lots were fed a diet containing 2.25 percent Ca and 0.6 percent P for eight weeks. At the end of the eight-week period each of the experimental diets fed in Trial 3 were assigned to two lots from each group of six lots as indicated above.

In this trial also five pullets from each lot were X-rayed before they were fed the experimental diet and at the end of each 28-day experimental period.

Method of Analysis

The data were statistically analyzed by the analysis of variance procedure according to Snedecor (1956). Significant mean differences were determined by Duncan's Multiple Range Test (1955).



IV. RESULTS AND DISCUSSION

Trial 1

Diets containing 1.50, 2.25, 3.00, 3.75, and 4.50 percent Ca with a Ca:P ratio of 3.75 to 1 were fed in this trial. The type of experimental design and diets used did not permit the effect of Ca and P levels to be separated.

Relatively few studies have been reported in which Ca:P ratios have been held constant. However, comparisons of the results of this trial with studies in which Ca:P ratios have been varied by changing the level of Ca or P appear to be justified and meaningful.

The average production of layers fed 1.50 percent Ca for the five experimental periods was significantly lower ($P \leq 0.05$ and 0.01) than that of layers fed diets with higher levels of Ca and P (Table IV) as determined by Duncan Multiple Range Test (1955). Pullets fed diets with 2.25 and 3.00 percent Ca performed similarly ($P > 0.05$); however, the egg production of pullets fed a diet with 3.00 percent Ca was as good as that of pullets fed diets containing 3.75 and 4.50 percent Ca ($P > 0.05$). At the 1 percent level of probability, pullets fed diets containing 2.25, 3.00, and 3.75 percent Ca laid at rates which failed to differ significantly.

The average egg production during period 1 was significantly higher ($P \leq 0.05$ and 0.01) than that of any of the other periods.



Table IV
Average Egg Production of Layers, Trial 1

Dietary Ca and P		Hen day egg production					Treatment average ²
		Period ¹					
		1	2	3	4	5	
Ca	P	percent					percent
-----percent-----		-----percent-----					-----percent-----
1.50	0.4	67.6	60.8	46.4	49.5	50.6	A _{54.9} ^{a2}
2.25	0.6	88.8	80.9	82.0	72.4	80.8	B _{81.4} ^b
3.00	0.8	91.2	84.0	82.0	82.5	80.4	BC _{84.4} ^{bc}
3.75	1.0	90.6	87.0	83.0	82.9	83.6	BC _{85.6} ^c
4.50	1.2	92.4	88.0	89.0	84.2	80.4	C _{87.6} ^c
Period average		^y 87.1 ^{z2}	^x 81.1 ^y	^x 77.8 ^{xy}	^x 75.0 ^x	^x 76.4 ^x	

¹Each period was 28 days in length.

²Means with different small letter superscripts within the column or line differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

Average egg production during periods 2 through 5 did not differ significantly at the 1 percent level of probability, but the production during period 2 did not differ from that of period 3, and that of period 3 did not differ from that of periods 4 and 5 at the 5 percent level of probability.

The results of this trial indicate that a level of no more than 3.0 percent Ca and 0.8 percent P may be required for egg production because higher levels of Ca and P failed to significantly affect production ($P > 0.01$).

It is possible that the Ca requirement for maximum egg production may be less than 3.00 percent of the diet since the egg production of birds fed the 2.25 and 3.00 percent Ca diets did not differ significantly ($P > 0.05$). It should be noted that during period 4, the rate of egg production of birds fed the 2.25 percent Ca diet was lower than that of layers fed the higher levels of Ca. This condition was not so marked in the other periods. Six of the 30 layers fed this diet almost ceased to lay for a period of over one week during period 4. The cause of this is not known. Had these birds continued to lay as they did at other times, it is quite possible that the production of birds on this diet would have been as good as that of birds fed the higher Ca diets. Of course, this may have been a treatment effect.

These results are in agreement with those of Miller and Bearse (1934), and Sanford and Alder (1969), but indicate a requirement of somewhat higher levels of dietary Ca than was suggested by Norris et al. (1934). It is possible that the 2.75 percent Ca level suggested

by the National Research Council (1971) may be adequate for maximum egg production.

The 1.50 percent Ca diet supported about 55 percent production during the experimental period. During periods 3, 4, and 5 the egg production of these birds tended to plateau near 50 percent while birds fed the other diets continued to lay at about 80 percent.

While the specific effect of dietary P levels on egg production cannot be separated from those of Ca, layers fed diets containing 0.8, 1.0, and 1.2 percent P laid at the same rate ($P > 0.05$). Thus, when Ca:P ratios of 3.75 to 1 were maintained high P levels had no apparent effect on egg production. This is not in agreement with Singen et al. (1962). However, these researchers did not maintain constant Ca:P ratios but increased P levels from 0.4 to 0.6 percent without increasing Ca levels in the diet.

The average egg specific gravity scores are given in Table V. The scores were significantly lower ($P \leq 0.05$ and 0.01) for eggs of layers fed 1.5 percent Ca than for eggs from hens fed diets containing the higher levels of Ca and P. The specific gravity score of eggs of layers fed 2.25 percent Ca was significantly higher ($P \leq 0.05$ and 0.01) than that of eggs from hens fed the lower level of Ca and P and was significantly lower ($P \leq 0.05$ and 0.01) than eggs from hens fed the higher levels of Ca and P. No difference ($P > 0.05$) was found in specific gravity score of the eggs from birds fed 3.0 percent Ca and 3.75 percent Ca. The specific gravity of eggs of layers fed 3.75, and 4.50 percent Ca did not differ ($P > 0.05$). There was no significant

Table V
Average Egg Specific Gravity Score of Layers, Trial 1

Dietary Ca and P Ca P -----percent-----		Average specific gravity score					Treatment average -----percent-----
		Period					
		1	2	3	4	5	
1.50	0.4	1.86	0.64	1.59	1.62	0.79	A _{1.30} ^{a2}
2.25	0.6	2.51	2.80	2.88	3.60	2.81	B _{2.92} ^b
3.00	0.8	4.44	4.42	4.12	4.44	3.98	C _{4.28} ^d
3.75	1.0	4.56	3.99	4.27	4.38	3.48	C _{4.14} ^{cd}
4.50	1.2	4.34	4.46	4.20	4.20	2.99	C _{4.04} ^c
Period average		YZ _{3.54} ^{z2}	Y _{3.26} ^y	YZ _{3.41} ^{yz}	Z _{3.65} ^z	X _{2.81} ^x	

¹Each period was 28 days in length.

²Means with different small letter superscripts within the column or line differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

difference ($P > 0.01$) in the specific gravity score of eggs from layers fed diets containing 3.0, 3.75, and 4.50 percent Ca.

The low level of Ca and P (1.50 percent Ca, and 0.4 percent P) reduced egg shell quality. This finding is in agreement with the conclusion of Wolford and Tanaka (1970) who stated that 18 authors agreed that less than 2 percent Ca in the diet significantly decreased egg shell quality. Hunt and Aitken (1962) also reported that 1.75 percent Ca level significantly depressed the specific gravity. The eggs of birds fed the diet containing 2.25 percent Ca were not of good shell quality. A similar finding was reported by Petersen et al. (1960) who stated that a level of 2.25 percent of Ca was not adequate for the production of eggs with the best shell quality.

Undoubtedly, the lower dietary levels of Ca had more effect on specific gravity scores of the eggs than did P since Walter and Aitken (1962) indicated that P levels in the range of 0.4 to 0.7 percent are satisfactory for good shell quality. These data tend to agree with those of Hunt and Chancey (1970) who reported that a reduction in the total dietary P from 0.72 to 0.39 percent had no effect on the shell quality of caged birds.

The bone density indexes are given in Table VI. The bone densitometer was inoperative during period 1 so bone density indexes could not be recorded at the end of this period. The bone density of pullets fed a diet with 1.50 percent Ca was significantly lower ($P \leq 0.05$ and 0.01) than that of birds fed diets with higher Ca levels. The bone density indexes of birds fed 2.25 and 3.00 percent Ca did not

Table VI
Average Bone Density Index of Left Tibia, Trial 1

Dietary Ca and P		Average bone density index						Treat- ment ² average
		Period ¹						
Ca	P	-1	0	2	3	4	5	
-----percent-----								
1.50	0.4	1.298	1.070	0.750 ^{a3}	0.802	0.800 ^{a3}	0.832 ^{a3}	A0.796 ^{a3}
2.25	0.6	1.385	1.158	0.986 ^{bc}	0.984	0.935 ^b	0.967 ^b	B0.968 ^b
3.00	0.8	1.254	1.123	0.938 ^b	1.007	0.958 ^{bc}	1.000 ^{bc}	B0.976 ^b
3.75	1.0	1.437	1.225	1.086 ^d	1.146	1.043 ^d	1.076 ^c	C1.088 ^d
4.50	1.2	1.254	1.186	1.021 ^c	1.043	1.018 ^c	1.056 ^{bc}	BC1.034 ^c
Period average		1.326 ⁴	1.152	0.956	0.996	0.951	0.986	

¹Each period was 28 days in length.

²Means of periods 2, 3, 4, and 5.

³Means with different small letter superscripts within the column or line differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

⁴Means for periods 2, 3, 4, and 5 did not differ significantly ($P > 0.05$).

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differ significantly ($P > 0.05$). The bone density of tibia of pullets fed a diet with 4.50 percent Ca was significantly lower ($P \leq 0.05$) than that of pullets fed a diet with 3.75 percent Ca but was significantly higher ($P \leq 0.05$) than the bone density of birds fed the other lower levels of dietary Ca in this trial. However, at the 1 percent level of probability there was no difference in bone density when birds were fed diets with 2.25, 3.00, and 4.50 percent Ca or with 3.75 and 4.50 percent Ca.

Averages for bone densities for periods 2, 3, 4, and 5 were analyzed individually. It was found that the diets significantly influenced these densities except in period 3. During period 2 significantly lower ($P \leq 0.05$) bone density was found in layers fed 1.50 percent Ca than in layers fed any of the higher levels of Ca and P. There was no significant difference in bone densities from layers fed 2.25, and 3.0 percent Ca. The bone densities from layers fed 2.25 and 4.50 percent Ca did not differ ($P > 0.05$). Birds fed 3.75 percent Ca and 1.0 percent P had a significantly higher ($P \leq 0.05$) average bone density than that of birds fed diets containing 1.5, 2.25, 3.00, and 3.75 percent Ca.

In period 4, the same pattern of bone densities were found as in period 2, except bone densities of layers fed 3.0 percent Ca did not differ from that of layers fed 4.50 percent Ca, while those of layers fed the 2.25 percent Ca were significantly lower ($P \leq 0.05$) than those of layers fed the 4.50 percent Ca diet.

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In period 5 the same pattern was found as in period 2 except that the bone densities of layers fed 4.50 percent Ca did not differ from that of layers fed 2.25, and 3.0 percent of Ca in the diet, and those of birds fed the 3.00, 3.75, and 4.50 percent Ca diets did not differ ($P > 0.05$).

The averages for periods 2, 3, 4, and 5 did not differ significantly ($P > 0.05$). This indicates that during the experimental period these bone densities tended to plateau.

The breaking strength of tibiae (Table VII) from layers fed a diet with 1.50 percent Ca was significantly lower ($P \leq 0.05$) than that of tibiae from layers fed diets with 3.00, 3.75, and 4.50 percent Ca. However, the breaking strength of tibiae of layers fed the diet containing 2.25 percent Ca did not differ significantly ($P > 0.05$) from those of layers fed diets containing 3.0 and 4.50 percent Ca. The breaking strengths of tibia of birds fed Ca levels of 3.0, 3.75, and 4.50 percent Ca did not differ significantly ($P > 0.05$).

The mean breaking strength of the tibiae increased as the level of dietary Ca and P increased up to 3.75 percent Ca in the diet. This result is in agreement with that of Rowland et al (1967a) who used 1.5, 3.0 (control) and 6.0 percent Ca in their diets. They did not mention the level of P used in their experiment.

Trial 2

A factorial design was used in this trial to determine the effect of different dietary Ca and P levels and ratios. Therefore,

Table VII
Average Breaking Strength of Left Tibiae, Trial 1

Dietary Ca and P		Breaking strength
Ca	P	
----percent----		----kilograms----
1.50	0.4	10.45 ^{a1}
2.25	0.6	15.20 ^{ab}
3.00	0.8	19.91 ^{bc}
3.75	1.0	25.21 ^c
4.50	1.2	22.61 ^c

¹Means with different small letter superscripts within the column differ significantly ($P \leq 0.05$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.



means were taken from each diet for the entire experimental periods and were analyzed statistically, except in the case of bone density index where analyses were made for periods 2, 3, 4, and 5 in addition to the mean of the periods. The bone densitometer was inoperative because of electronic malfunction so bone density indexes could not be determined at the end of period 1.

The average egg production by periods of layers fed 3.0, and 3.5 percent Ca and different levels of P is given in Table VIII. The various Ca levels in the experimental diets used in this study provided no statistically important effect. Statistical analyses of the average egg production were made at the end of the trial to determine the effect of the various diets after they had been fed for five 28-day periods.

The egg production of layers fed 0.4 percent P diets was significantly lower ($P \leq 0.05$ and 0.01 , Table IX) than that of layers fed 0.6, 0.8, and 1.0 percent P. At the 1 percent level of probability no significant difference in egg production of layers fed 0.4 and 1.2 percent P in the diet was observed. The egg production of layers fed 0.6 percent P diets was significantly higher ($P \leq 0.05$) than that of layers fed diets containing 0.4, 0.8, 1.0, and 1.2 percent P, but did not differ significantly from the egg production of hens fed diets containing 1.0 percent P at the 1 percent level of probability.

When the average egg production at the end of each period was considered (Table VIII), the egg production at the end of period 1 was significantly higher ($P \leq 0.05$ and 0.01) than that of periods 3, 4,

Table VIII

Average Egg Production of Layers, Trial 2

Dietary Ca and P		Average egg production				
		Period ¹				
Ca	P	1	2	3	4	5
-----percent-----		-----percent-----				
3.0	0.4	86.8	83.3	77.4	77.1	79.5
	0.6	88.7	86.7	88.5	83.2	83.2
	0.8	89.8	86.7	83.7	82.7	82.9
	1.0	89.1	86.9	86.4	81.2	76.2
	1.2	87.8	84.8	79.5	79.3	76.8
3.5	0.4	86.6	83.2	80.2	78.3	74.7
	0.6	91.1	88.7	83.3	86.4	85.4
	0.8	86.0	84.6	82.0	77.6	77.9
	1.0	88.6	87.3	86.0	79.5	78.5
	1.2	89.2	87.4	85.0	81.4	79.2
Period average		Z _{88.4} ^z ²	YZ _{86.0} ^y	XY _{83.6} ^x	WX _{80.8} ^w	W _{79.5} ^w

¹Each period was 28 days in length.

²Means with different small letter superscripts within the line differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

Table IX
Summary of Data from Trial 2

Dietary P and Ca -percent-	Egg production --percent-	Specific gravity score	Bone density -index-	Bone breaking strength kilograms
<u>Phosphorus</u>				
0.4	A _{80.50} ^{a1}	B _{4.35} ^{c1}	A _{0.955} ^{a1}	26.15 ²
0.6	C _{86.80} ^c	B _{4.14} ^b	AB _{1.000} ^b	25.83
0.8	B _{83.60} ^b	B _{4.17} ^b	BC _{1.039} ^c	25.07
1.0	BC _{84.20} ^b	A _{3.84} ^a	C _{1.076} ^c	27.37
1.2	AB _{83.30} ^b	A _{3.89} ^a	BC _{1.042} ^c	26.12
<u>Calcium</u>				
3.0	83.80 ²	4.05	1.014	26.35
3.5	83.80 ²	4.11	1.031	25.87

¹Means with different small letter superscripts within the column differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

²Means with no superscripts within the column or line did not differ significantly ($P > 0.05$).

and 5. No significant difference ($P > 0.01$) in average egg production occurred when the following different trial periods were compared:

(1) period 1 with period 2; (2) period 2 with period 3; (3) period 3 with period 4; and (4) period 4 with period 5.

At the 5 percent level of probability the average egg production during the period 1 was significantly higher than that during periods 2, 3, 4, and 5; the average egg production during period 2 was also higher than that of period 3, and the average egg production during period 3 was higher than that of periods 4 and 5. The average egg production during periods 4 and 5 was not significantly different ($P > 0.05$).

The results of this study have been interpreted as an indication that the most suitable dietary levels of P and Ca for egg production was about 0.6 percent P when 3.0 or 3.5 percent Ca was used. In trial 1, egg production was significantly higher ($P \leq 0.01$) for layers fed the 3.0 percent Ca and 0.8 percent P diet than for layers fed the lower levels of dietary Ca and P but did not differ from that of birds fed diets containing 3.75 percent Ca and 1.0 percent P or 4.5 percent Ca and 1.2 percent P. In this trial when the P levels were increased to 0.8, 1.0, and 1.2; in diets containing 3.0 or 3.5 percent Ca, a decrease in egg production occurred. In trial 1, the ratio of 3.75:1 for dietary Ca and P levels was maintained, therefore, in trial 2 hens fed diets with Ca:P ratios of 2.50:1 to 3.75:1 (diets containing 3.0 or 3.5 percent Ca and 0.8, 1.0, and 1.2 percent P) had lower production.

Based on the results of trials 1 and 2 it appears that ca:P ratios greater than 3.75:1 may be more desirable.

In this trial, it was also noted that the age of layers had considerable influence on egg production. The layers showed the highest egg production at the beginning of the experiment followed by a gradual decrease in the rate of egg production as the birds became older.

Egg specific gravity scores for layers fed 0.4 percent P diets were significantly higher ($P \leq 0.05$) than those for birds fed diets containing all other higher levels of P (Table IX). The egg specific gravity scores for birds receiving (1) 0.6 and 0.8 percent P; and (2) 1.0 and 1.2 percent P, revealed no comparative significant difference within each group ($P > 0.05$). The average specific gravity score (Table X) was not significantly different ($P > 0.05$ and 0.01) at the end of periods 1 and 4 when all mean scores of each period were pooled and averaged. The comparative average specific gravity for periods 2 and 3, and for periods 2 and 4 did not differ significantly ($P > 0.01$). The average specific gravity score for period 5 was found to be significantly lower ($P < 0.05$) than the averages for periods 1, 2, 3 and 4. At the 5 percent level of probability, the egg specific gravity means for periods 1 and 4, as well as for periods 2 and 3, showed no significant difference.

From the standpoint of specific gravity the finest egg shell quality was obtained from birds fed a diet containing 0.4 percent P, a result in agreement with that obtained by Walter and Aitken (1962). However, these had the lowest rate of production and this may have been

Table X
Average Egg Specific Gravity Score, Trial 2

Dietary Ca and P Ca P		Average specific gravity score				
		Period ¹				
-----percent-----		1	2	3	4	5
3.0	0.4	4.51	3.92	4.57	5.05	4.18
	0.6	4.00	3.86	3.80	4.15	3.70
	0.8	4.65	4.39	4.14	4.47	3.46
	1.0	4.23	4.06	3.70	3.59	3.93
	1.2	3.94	3.68	3.84	3.84	3.50
3.5	0.4	4.78	4.52	4.01	4.22	3.77
	0.6	4.70	4.50	4.34	4.67	3.66
	0.8	4.50	3.92	4.07	4.30	3.78
	1.0	3.96	4.01	3.34	3.96	3.66
	1.2	4.68	3.89	3.69	4.64	3.16
Period average		Z _{4.40} ^{y2}	XY _{4.08} ^x	WX _{3.95} ^x	YZ _{4.29} ^y	W _{3.68} ^w

¹Each period was 28 days in length.

²Means with different small letter superscripts within the line differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

a factor in improving shell quality. In comparing the results of trials 1 and 2, in trial 1 the specific gravity scores of eggs from layers fed 0.8 and 1.0 percent P diets were significantly higher ($R \leq 0.05$) than those of eggs from layers fed the 0.4 and 0.6 percent of P diets.

In trial 1, a dietary level of 0.4 percent P failed to produce as high egg specific gravity scores as it did in trial 2. The reason for this low specific gravity of eggs in trial 1 probably was caused by the very low level of Ca (1.5 percent).

The average bone density indexes of the left tibiae are reported in Table XI. They were taken at six different times: (1) one period interval prior to the experiment; (2) at the beginning of period 1; and (3) at the end of periods 2, 3, 4, and 5. The bone density indexes of pullets fed diets with 0.8, 1.0, and 1.2 percent P did not differ significantly ($P > 0.05$), but were significantly higher ($P \leq 0.05$) than the indexes of layers fed the 0.4 or 0.6 percent P diets (Table IX, page 37). Layers fed 0.4 percent P had significantly lower bone density indexes ($R \leq 0.05$) than that of all other higher levels of dietary P. The bone density indexes of layers fed 0.6 percent P were significantly higher ($P \leq 0.05$) than those of layers fed 0.4 percent P, and significantly lower ($P \leq 0.05$) than those of the layers fed higher levels of P in their diet. At the 1 percent level of probability, bone densities from layers fed 0.4 and 0.6 percent P did not differ, nor did the bone density of those layers fed 0.6, 0.8, and 1.2 percent P and no significant difference was found in bone densities of layers fed 0.8 percent P diet did not differ from that of kinds fed the 1.0 percent P diet,

Table XI
Average Bone Density Index of Left Tibia, Trial 2

Dietary Ca and P Ca P -----percent-----		Average bone density index					
		Period ¹					
		-1	0	2	3	4	5
3.0	0.4	1.327	1.232	0.988	0.865	0.946	0.965
	0.6	1.448	1.228	0.933	0.992	0.996	0.984
	0.8	1.277	1.258	1.057	1.021	1.049	1.074
	1.0	1.249	1.286	1.014	1.104	1.144	1.020
	1.2	1.295	1.185	0.969	0.996	1.086	1.078
3.5	0.4	1.319	1.087	0.950	0.962	1.034	0.929
	0.6	1.261	1.133	0.966	1.012	1.040	1.073
	0.8	1.116	1.107	1.000	1.032	1.116	0.967
	1.0	1.246	1.255	1.014	1.066	1.176	1.076
	1.2	1.286	1.110	0.986	1.078	1.135	1.005
Period average		1.282	1.188	^W 0.988 ^{w2}	^W 1.013 ^w	^X 1.072 ^x	^W 1.017 ^w

¹Each period was 28 days in length.

²Means of periods 2 through 5 with different small letter superscripts within the same line differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

and the bone density of layers fed 1.0 percent P diet did not differ from that of birds fed 1.2 percent P diet.

At the end of each period the means were averaged (Table XI). The averages of the bone density index of periods 2, 3, and 5 did not differ significantly ($P > 0.05$ and 0.01). At the 1 and 5 percent level of probability the average bone density index of period 4 was significantly higher than that of periods 2, 3, and 5.

To determine how quickly the treatment effect appeared the period means for each treatment were analyzed by period as shown in Table XII. Treatment effects were not significantly different for periods 3 and 5.

In period 2, the bone density indexes from birds fed 0.4, 0.6, and 1.2 percent P did not differ significantly ($P > 0.05$ and 0.01). At the 5 percent level of probability, the bone density indexes from birds fed 0.4, 0.6, and 1.2 percent P were significantly lower than for birds fed 0.8 percent P. Bone density indexes from birds fed 0.8 percent P diet did not differ, 1.0, and 1.2 percent P did not differ ($P > 0.05$). At the 1 percent level of probability the bone density of the layers fed a diet with 0.4 percent P did not differ significantly with that of layers fed 1.0 and 1.2 percent P. The bone density index of layers fed 1.2 percent P did not differ significantly with that of layers fed 1.0 or 0.8 percent P ($P > 0.01$).

In period 4, the bone density indexes of layers fed 0.4, 0.6, and 0.8 percent P did not differ significantly ($P > 0.05$) and those of layers fed 0.8, 1.0, and 1.2 percent P did not differ significantly

Table XII

Effect of Dietary Phosphorus on Bone Density Index, Trial 2

Dietary p ¹ --percent--	Average bone density index	
	Period 2	Period 4
0.4	AB 0.967 ^{a2}	0.990 ^{a1}
0.6	A 0.950 ^a	1.018 ^a
0.8	C 1.029 ^c	1.083 ^{ab}
1.0	BC 1.014 ^{bc}	1.160 ^b
1.2	ABC 0.977 ^{ab}	1.111 ^b

¹Each phosphorus level was fed with 3.0 and 3.50 percent Ca in the diet.

²Means with different small letter superscripts within the column differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

($P > 0.05$). The bone density indexes of layers fed 0.4 and 0.6 percent P had significantly lower bone density indexes ($P \leq 0.05$) than those birds fed 1.0 and 1.2 percent P.

It should be noted that the average specific gravity score of eggs for period 4 of trial 2 was higher than the averages of periods 2, 3, or 5; and the bone density index was also higher for period 4 in this trial than the averages of periods 2, 3, or 5.

The most suitable level of P for bone density index was about 0.8 percent. From this trial, it also appears that layers, before the experiment started, had higher bone density indexes, but when they were fed the experimental diet and were in peak of lay, they tended to maintain a certain level of bone density. It also appears that in period 2 diets have more influence on bone density index for as the laying period increased, this variability in density became less. In trial 1 also the layers had higher bone density indexes before the experiment started, but afterward they tended to maintain a certain level. In trial 1, 0.8 percent P did not perform as well as in this trial as far as bone density was concerned. In trial 1 the highest bone density index of layers appeared when the layers were fed 3.75 percent Ca and 1.0 percent P, which indicates that if the P level is raised from 0.8 percent, the Ca level also must be increased.

The breaking strength of the tibiae is given in Table XIII. The different levels of Ca and P used in this trial failed to significantly affect bone breaking strength at the 5 percent level of

Table XIII
Average Breaking Strength of Left Tibiae, Trial 2

Dietary Ca and P		Breaking strength
Ca	P	
----percent----		---kilograms---
3.0	0.4	28.49
	0.6	26.87
	0.8	24.00
	1.0	25.62
	1.2	26.75
3.5	0.4	23.83
	0.6	24.79
	0.8	26.15
	1.0	29.11
	1.2	25.49

probability. The great variation in bone breaking strength in replicates is hard to explain under the experimental conditions of this study.

Trial 3

This trial was designed to determine the effect of feeding diets containing 1.50, 2.25, and 3.00 percent Ca with 0.4, 0.6, and 0.8 percent P, respectively, when the diets were first fed to layers at 21 weeks of age as measured by egg production, specific gravity, bone density, and bone breaking strength. Therefore, statistical analyses were done on data obtained in the last period of the trial except for bone density index, where the interest was to determine just how quickly the diets affected the bone density of these layers.

The average egg production of the layers is given in Table XIV. The egg production of layers fed the diet containing 1.5 percent Ca and 0.4 percent P for 16 weeks was significantly lower at the 5 percent level of probability than that of layers fed the same diet for 12 or eight weeks, however, there was no significant difference in egg production of layers fed a diet containing the above levels of Ca and P for 16, 12 or eight weeks at the 1 percent level of probability. The egg production of layers fed a diet containing 1.5 percent Ca and 0.4 percent P for a period of 12 or eight weeks was not significantly different ($P > 0.05$). The egg production of layers fed a diet containing 2.25 percent Ca and 0.6 percent P for a period of 16, 12, or eight weeks and that of layers fed 3.0 percent Ca and 0.8 percent P for 16, 12, or eight weeks did not differ significantly ($P > 0.05$).

Table XIV
Average Egg Production of Layers, Trial 3

						Average egg production			
Period 1		Period 2		Periods 3 and 4		Period			
Ca	P	Ca	P	Ca	P	1	2	3	4
-----percent-----						-----percent-----			
1.50	0.4	1.50	0.4	1.50	0.4	61.54	68.81	62.40	A ^{53.95} ^{a1}
2.25	0.6	2.25	0.6	2.25	0.6	49.52	79.42	81.51	B ^{79.34} ^c
3.00	0.8	3.00	0.8	3.00	0.8	64.64	82.24	90.70	B ^{84.40} ^c
3.00	0.7	1.50	0.4	1.50	0.4	51.67	70.20	64.32	A ^{64.81} ^b
		2.25	0.6	2.25	0.6	61.22	86.62	89.09	B ^{80.58} ^c
		3.00	0.8	3.00	0.8	62.38	85.12	85.84	B ^{77.26} ^c
3.00	0.7	3.00	0.7	1.50	0.4	67.74	76.67	75.96	A ^{61.90} ^b
				2.25	0.6	57.62	77.26	87.49	B ^{80.12} ^c
				3.00	0.8	68.10	87.62	88.81	B ^{81.78} ^c

¹Means with different small letter superscripts within the column differ significantly ($R \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$); as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

These findings agree with the results found in trial 1 where the egg production of layers fed a diet with 1.5 percent Ca and 0.4 percent P was significantly lower ($P \leq 0.05$ and 0.01) than that of layers fed a diet containing either 2.25 percent Ca and 0.6 percent P or 3.0 percent Ca and 0.8 percent P, and the egg production of layers fed 2.25 percent Ca, 0.6 percent P, and 3.0 percent Ca, 0.8 percent P did not differ significantly ($P > 0.05$). However, trial 1 covered a length of 20 weeks of experimental time. In trial 2 at the 5 percent level of probability the egg production of layers fed a level of 3.0 percent Ca and 0.6 percent P diet was significantly higher than that of birds fed either 0.4, 0.8, 1.0, or 1.2 percent P. It is possible that in this trial hens fed a diet with the 0.6 percent P failed to lay at a significantly higher rate than hens fed a diet with 0.8 percent P because of the lower level of Ca fed with this P level.

In this trial the diet containing 1.5 percent Ca and 0.4 percent P supported 50 percent egg production during period 4. This is comparable to the results obtained in trial 1.

The average egg specific gravity score is given in Table XV. The egg specific gravity score of layers fed a diet containing 1.5 percent Ca and 0.4 percent P for 16, 12, or eight weeks was significantly lower ($P \leq 0.05$) than the scores of eggs from birds fed higher levels of Ca and P. The egg specific gravity scores of birds fed higher dietary levels of Ca and P (2.25 percent Ca, 0.6 percent P; and 3.0 percent Ca, 0.8 percent P) did not differ significantly ($P > 0.05$) in the fourth experimental period even though these diets had been fed for 16, 12, or eight weeks.

Table XV
Average Egg Specific Gravity Score of Layers, Trial 3

Ca and P level in diet						Average egg specific gravity score			
Period 1		Period 2		Periods 3 and 4		Period			
Ca	P	Ca	P	Ca	P	1	2	3	4
-----percent-----						-----score-----			
1.50	0.4	1.50	0.4	1.50	0.4	1.72	2.10	1.38	A _{0.72} ^{a1}
2.25	0.6	2.25	0.6	2.25	0.6	3.88	4.76	3.22	BC _{2.90} ^b
3.00	0.8	3.00	0.8	3.00	0.8	4.07	5.08	3.22	C _{3.00} ^b
3.00	0.7	1.50	0.4	1.50	0.4	4.37	2.09	2.04	AB _{1.34} ^a
		2.25	0.6	2.25	0.6	3.68	4.22	3.23	BC _{2.64} ^b
		3.00	0.8	3.00	0.8	4.09	5.10	4.07	BC _{2.84} ^b
3.00	0.7	3.00	0.7	1.50	0.4	4.48	5.10	1.28	A _{0.73} ^a
				2.25	0.6	4.25	4.88	2.94	BC _{2.58} ^b
				3.00	0.8	4.50	5.06	3.52	C _{3.51} ^b

¹Means with different small letter superscripts within the column differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

In trial 1, egg specific gravity score of birds fed 2.25 percent Ca and 0.6 percent P was significantly lower ($P \leq 0.05$) than that of birds fed with 3.0 percent Ca and 0.8 percent P, while in trial 3 there was no significant difference in egg specific gravity from birds fed either 2.25 percent Ca, 0.6 percent P; or 3.0 percent Ca, 0.8 percent P. It may be explained that trial 1 was conducted during the summer and fall months while this trial was run in the winter and spring. The colder weather of the winter and spring months may have reduced somewhat the bird's Ca requirement, thereby eliminating the response to high levels of Ca. In trial 1, the layers were fed the diet for 20 weeks but in this trial they were fed the diet only for 16, 12, or eight weeks. It seems probable that layers in this trial were also using their reserve Ca during 16, 12, or eight weeks. The critical period of this reserve may be somewhere between 16 and 20 weeks.

Between periods 3 and 4 a general decrease in egg specific gravity was found regardless of the diet fed. This difference may be explained by indicating that period 3 was run in the month of April when the environment was moderately warm, and period 4 was run in the month of May when the temperature was warmer than that of period 3. This difference of temperature may have been high enough to lower the egg specific gravity score. This theory is supported by the findings of Conrad (1933), Frøning and Funk (1958), Tyler and Greake (1958), and Durham et al. (1960). The lowering in egg specific gravity by the higher temperature might have been aggravated by the age of the layers.

The egg specific gravity score of all groups of layers transferred from a diet containing 3.0 percent Ca and 0.7 percent P to a diet containing 1.5 percent Ca and 0.4 percent P decreased markedly during the first four weeks they were fed the diet. This indicates that when layers are transferred from a higher dietary level of Ca and P to a deficient level of Ca and P, the egg specific gravity decreased rather quickly.

The bone density indexes for the trial 3 are given in Table XVI. In period 3, the bone density indexes of layers fed a diet with 1.5 percent Ca and 0.4 percent P for 12, eight or four weeks did not differ significantly ($P > 0.05$). The bone density indexes of layers fed a diet containing 2.25 percent Ca and 0.6 percent P for 12 and four weeks did not differ significantly ($P > 0.05$). The bone density indexes of layers fed the 2.25 percent Ca and 0.6 percent P diet for 12 weeks was significantly lower ($P \leq 0.05$) than that of layers fed the same diet for eight weeks. At the 5 percent level of probability layers fed with 2.25 percent Ca and 0.6 percent P for four weeks did not differ significantly with that of layers fed the same diet for eight weeks. The bone density indexes of layers fed a diet with 2.25 percent Ca and 0.6 percent P for 12, eight, or four weeks did not differ significantly at the 1 percent level of probability. The bone density indexes of layers fed a diet with 2.25 percent Ca and 0.6 percent P for 12, eight, or four weeks and that of layers fed 3.0 percent Ca, 0.8 percent P for the same duration of time did not differ significantly ($P > 0.01$).

Table XVI
Average Bone Density Index of Left Tibia of Layers, Trial 3

Average bone density index						
Period 1		Period 2		Periods 3 and 4		
Ca	P	Ca	P	Ca	P	
percent						
1.50	0.4	1.50	0.4	1.50	0.4	
2.25	0.6	2.25	0.6	2.25	0.6	
3.00	0.8	3.00	0.8	3.00	0.8	
3.00	0.7	1.50	0.4	1.50	0.4	
		2.25	0.6	2.25	0.6	
		3.00	0.8	3.00	0.8	
3.00	0.7	3.00	0.7	1.50	0.4	
				2.25	0.6	
				3.00	0.8	
Average bone density index						
Period		0	1	2	3	4
Percent						
1.127						
0.882						
0.886						
A ^{0.840a}						
A ^{0.810a¹}						
1.124						
0.905						
0.928						
ABC ^{0.926ab}						
AB ^{0.921bc}						
1.116						
1.052						
0.998						
BC ^{1.006c}						
B ^{0.972bcd}						
1.129						
1.050						
0.886						
AB ^{0.901a}						
AB ^{0.902ab}						
1.122						
1.060						
0.994						
BC ^{1.014c}						
B ^{0.974bcd}						
1.220						
1.062						
1.050						
BC ^{1.020c}						
B ^{1.010d}						
1.142						
1.004						
0.974						
A ^{0.859a}						
A ^{0.832ab}						
1.186						
1.114						
1.046						
BC ^{0.996bc}						
B ^{0.991cd}						
1.086						
1.022						
1.032						
C ^{1.027c}						
B ^{0.984bcd}						

¹Means with different small letter superscripts within the column differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P < 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

At the 1 percent level of probability it appears that the feeding of each of these experimental diets for 12, eight, or four weeks had no influence on the bone density indexes of layers. At the 5 percent level of probability the same result was observed except the bone density index of layers fed with Ca 2.25 percent and 0.6 percent P for eight weeks which was significantly higher ($P \leq 0.05$) than that of layers fed the same diet for 12 weeks. The change of diet shows its effect on bone density indexes quickly.

In period 4, the bone density index of layers fed a diet with 1.5 percent Ca and 0.4 percent P did not differ significantly ($P > 0.05$) even though some of the birds had been fed this diet for 16, 12, or eight weeks. The bone indexes of layers fed a diet containing 2.25 percent Ca and 0.6 percent P did not differ significantly ($P > 0.05$) and that of layers fed 3.0 percent Ca and 0.8 percent P for 16, 12, or eight weeks also did not differ significantly ($P > 0.05$). At the 1 percent level of probability the bone density indexes of layers fed a diet containing 2.25 percent Ca and 0.6 percent P did not differ significantly with that of layers fed a diet containing 3.0 percent Ca and 0.8 percent P for 16, 12, or eight weeks.

The breaking strengths of tibiae are given in Table XVII. There was no significant difference ($P > 0.05$) in breaking strength of tibiae of layers fed any experimental diet for 16, 12, or eight weeks. There was considerable variability in the breaking strength of replicates; however, there was a trend for the higher levels of dietary Ca and P to increase breaking strength.



Table XVII

Average Breaking Strength of Tibiae, Trial 3

Ca and P level in diet						Average breaking strength kilogram
Period 1		Period 2		Periods 3 and 4		
Ca	P	Ca	P	Ca	P	
percent						
1.50	0.4	1.50	0.4	1.50	0.4	13.92 ^{ab1}
2.25	0.6	2.25	0.6	2.25	0.6	18.46 ^{abc}
3.00	0.8	3.00	0.8	3.00	0.8	24.36 ^c
3.00	0.7	1.50	0.4	1.50	0.4	19.48 ^{abc}
		2.25	0.6	2.25	0.6	22.51 ^{bc}
		3.00	0.8	3.00	0.8	27.53 ^c
3.00	0.7	3.00	0.7	1.50	0.4	12.68 ^a
				2.25	0.6	20.68 ^{abc}
				3.00	0.8	26.91 ^c

¹Means with different superscripts within the column differ significantly ($P \leq 0.10$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

At the 10 percent level of probability the breaking strength of tibiae of birds fed 1.5 percent Ca and 0.4 percent P for the last two periods was significantly lower than those fed 3.0 percent Ca and 0.8 percent P for 16, 12, or eight weeks, and that of layers fed the 2.25 percent Ca, 0.6 percent P diet for 12 weeks.

From these variations it may be concluded that the reasonably good diets fed to the layers before the experimental diet had no influence on the response of the layers to this criterion of study after they had been fed the experimental diets for eight, 12 or 16 weeks.

Trial 4

This trial was designed to determine the effect of different levels of Ca and P in the diet during the time the layers were 21 to 29 weeks old on the response of the layers to lower and/or higher levels of Ca and P in the diet during an ensuing eight-week experimental period. Therefore, only the data for period 4 were analyzed except for that of bone density index for which data for both periods 3 and 4 were analyzed to determine how soon the diets affected the bone density of these layers.

The egg production of birds fed the 3.0 percent Ca diet during periods 1 and 2 and the 1.50 percent Ca diet during periods 3 and 4 did not differ significantly ($P > 0.05$) with that of layers fed the 2.75 and 2.25 percent levels of Ca in periods 1 and 2 and 1.50 percent Ca diet in periods 3 and 4 (Table XVIII). The layers fed 3.0 percent Ca diet during periods 1 and 2 and the 2.25 percent Ca diet during

Table XVIII
Average Egg Production of Layers, Trial 4

Ca and P levels of diets				Average egg production			
Periods 1 and 2		Periods 3 and 4		Period			
Ca	P	Ca	P	1	2	3	4
percent				percent			
3.00	0.7	1.50	0.4	67.74	76.67	75.96	AB _{61.90} ^{ab}
		2.25	0.6	57.62	77.26	87.49	C _{80.12} ^c
		3.00	0.8	68.10	87.62	88.81	C _{81.78} ^c
2.75	0.6	1.50	0.4	60.95	86.07	67.56	A _{55.56} ^a
		2.25	0.6	47.86	74.76	75.46	B _{69.28} ^b
		3.00	0.8	54.05	82.26	87.86	C _{78.48} ^c
2.25	0.6	1.50	0.4	59.41	80.84	70.20	A _{57.80} ^a
		2.25	0.6	49.52	79.42	81.51	C _{79.34} ^c
		3.00	0.8	60.00	80.00	92.74	C _{85.92} ^c

¹Means with different small letter superscripts within the column differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

periods 3 and 4 did not differ significantly ($P > 0.05$) with that of layers fed a 2.25 percent Ca diet during periods 1, 2, 3, and 4. The egg production of layers fed a diet containing 2.75 percent Ca in periods 1 and 2 and 2.25 percent Ca diet in periods 3 and 4 was significantly lower ($P \leq 0.05$ and 0.01) than that of layers fed the lower and higher Ca level diets in periods 1 and 2 and a diet containing 2.25 percent Ca during periods 3 and 4. The layers fed a diet containing 3.0 percent Ca in periods 1 and 2 and 2.25 percent Ca in periods 3 and 4, and layers fed a 2.25 percent Ca diet for periods 1, 2, 3, and 4 did not differ significantly ($P > 0.05$) with that of layers fed a diet containing 3.0 percent Ca and 0.8 percent P in periods 3 and 4 regardless of the diets they were fed in periods 1 and 2. The layers fed the 2.75 percent Ca diet in periods 1 and 2, and the 2.25 percent Ca diet in periods 3 and 4 had significantly lower ($P \leq 0.05$ and 0.01) egg production than did layers fed the same level of Ca or higher level of Ca diet in periods 3 and 4.

Examination of the egg production data for the birds fed the 2.75 percent Ca diet in periods 1 and 2 and the 2.25 percent Ca diet in periods 3 and 4 revealed that two birds out of 30 on this diet did not lay any eggs during the entire period, therefore, the percentage of egg production was low. The egg production of layers fed a diet containing 2.25 percent Ca, 0.6 percent P was as good as that of layers fed 3.0 percent Ca and 0.8 percent P except that the group of layers fed 2.75 percent Ca diet in periods 1 and 2, and a diet containing 2.25 percent Ca in periods 3 and 4.

The result showed that the egg production of layers fed a diet containing 1.50 percent Ca and 0.4 percent P was not as adversely affected in period 3, just after they had been transferred from the 3.0 percent Ca and 0.7 percent P diet as was the egg production of layers fed the same experimental diet but fed either 2.75 or 2.25 percent Ca diet in periods 1 and 2. It appears that there may have been a slight carry over effect of the higher level of dietary Ca fed in periods 1 and 2 into period 3 for layers fed the lower levels of Ca during period 3. It should be noted that the egg production of layers fed a diet containing 2.75 percent Ca in periods 1 and 2 and 2.25 percent Ca diet during periods 3 and 4 was low in period 4.

The average egg specific gravity score is given in Table XIX. The layers fed the 2.25 percent Ca diet during periods 1 and 2, and the 3.0 percent Ca diet during periods 3 and 4 laid eggs during period 4 with higher specific gravity scores than did birds fed the same diet during periods 3 and 4 but which had been fed a diet containing 2.75 percent Ca during periods 1 and 2 ($P \leq 0.05$ and 0.01). The difference between the scores for the birds originally fed the 2.25 and 3.0 percent Ca diets and layers fed the 3.0 percent Ca diet during periods 3 and 4 was not significantly different ($P > 0.05$). Likewise, the difference between the scores for the birds originally fed the 3.0 and 2.75 percent Ca diets and the 3.0 percent Ca diet in periods 3 and 4 did not differ significantly ($P > 0.05$). Layers fed the 2.25 percent Ca diet during periods 3 and 4 laid eggs during period 4 which had similar specific gravity scores ($P > 0.05$). The specific gravity scores of eggs of

Table XIX

Average Egg Specific Gravity Score of Layers, Trial 4

Ca and P levels of diets				Average specific gravity			
Periods 1 and 2		Periods 3 and 4		Period			
Ca	P	Ca	P	1	2	3	4
percent				score			
3.00	0.7	1.50	0.4	4.48	5.10	1.28	A _{0.73} ^{a1}
		2.25	0.6	4.25	4.88	2.94	B _{2.43} ^b
		3.00	0.8	4.50	5.06	3.52	CD _{3.51} ^{de}
2.75	0.6	1.50	0.4	4.82	5.08	0.70	A _{0.58} ^a
		2.25	0.6	4.93	5.00	2.00	B _{2.43} ^b
		3.00	0.8	4.60	4.83	4.01	BC _{3.12} ^{cd}
2.25	0.6	1.50	0.4	4.38	5.14	0.62	A _{0.44} ^a
		2.25	0.6	3.88	4.76	3.22	BC _{2.90} ^{bc}
		3.00	0.8	4.44	5.16	4.45	D _{3.72} ^e

¹Means with different small letter superscripts within the column differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

layers fed the 1.50 percent Ca diet were significantly lower ($P \leq 0.05$ and 0.01) than those of layers fed higher levels of Ca.

Period 3 of this trial occurred in April and the period 4 in the month of May when the environment was warmer in comparison to that of April. There was a decrease in egg specific gravity score for all groups of layers between periods 2 and 3, the decrease being most marked in birds fed the lowest levels of Ca. Between periods 3 and 4 most of the groups experienced a further decrease in egg specific gravity score but some of the groups tended to plateau. This decrease in egg specific gravity score was associated with the rise of environmental temperature but the age of these layers may have aggravated this effect. In trial 3 such a decrease in egg shell quality was also observed. This phenomenon and the reports in the literature were related earlier in discussing the results of trial 3.

There was a greater decrease in egg specific gravity score when layers fed either 3.0, 2.75, or 2.25 percent Ca diet in periods 1 and 2 were fed diet containing 1.5 percent Ca than when layers were fed 3.00 or 2.25 percent Ca diet in period 3. A further decrease in the same pattern as was found in period 3 of this trial was observed in the egg specific gravity score of layers in period 4.

The bone density indexes for the periods 0, 1, 2, 3, and 4 are given in Table XX. In period 3, the bone density index of layers fed a diet containing 1.50 percent Ca did not differ significantly ($P > 0.05$). The bone density index of layers fed a diet containing 3.0, and 2.75 percent Ca in periods 1 and 2, and 3.0 percent Ca in period 3 were

Table XX

Average Bone Density Index of Left Tibia of Layers, Trial 4.

Ca and P levels of diets				Average bone density index				
Periods 1 and 2		Periods 3 and 4		Period				
Ca	P	Ca	P	0	1	2	3	4
-----percent-----								
3.00	0.7	1.50	0.4	1.142	1.004	0.974	A _{0.859} ^{ab}	A _{0.834} ^{ab} ¹
		2.25	0.6	1.186	1.114	1.046	B _{0.996} ^{cd}	B _{0.991} ^{cd}
		3.00	0.8	1.086	1.022	1.032	B _{1.027} ^d	B _{0.984} ^{cd}
2.75	0.6	1.50	0.4	1.094	1.026	1.021	A _{0.821} ^a	A _{0.814} ^a
		2.25	0.6	1.101	1.064	1.032	B _{0.972} ^{cd}	B _{0.987} ^{cd}
		3.00	0.8	1.166	1.104	1.065	B _{1.024} ^d	B _{1.027} ^d
2.25	0.6	1.50	0.4	1.128	0.968	0.938	A _{0.827} ^a	A _{0.798} ^a
		2.25	0.6	1.124	0.905	0.928	AB _{0.926} ^{bc}	AB _{0.921} ^{bc}
		3.00	0.8	1.103	0.975	0.895	AB _{0.920} ^{bc}	AB _{0.930} ^{cd}

¹Means with different small letter superscripts within the column differ significantly ($P \leq 0.05$); those with different capital letter superscripts differ significantly ($P \leq 0.01$) as determined by D. B. Duncan, 1955. Multiple range and multiple F test. Biometrics, 11:1-42.

significantly higher ($P \leq 0.05$) than those of layers fed a diet containing 2.25 percent Ca in period 1 and 2 and 3 percent Ca in period 3. At the 5 percent level of probability the bone density indexes of layers fed a diet containing 3.0 percent Ca in period 1 and 2; and a diet containing either 2.25 or 3.0 percent Ca in periods 3 and 4 and the bone density indexes of layers fed a diet containing 2.75 percent Ca in periods 1 and 2, and 2.25 and 3.0 percent Ca in periods 3 and 4 did not differ.

At the 1 percent of probability no significant difference was observed in layers fed 1.5 percent Ca diet during period 3 regardless of the levels of Ca and P in the diet fed in periods 1 and 2; that of layers fed 2.25 percent Ca diet in period 3 did not differ significantly with that of layers fed 3.0 percent Ca in period 3 regardless of the levels of Ca and P in the diets in periods 1 and 2.

However, the bone density index of birds fed the 2.25 percent Ca diet during periods 1 and 2 and the 2.25 and 3.0 percent Ca diets in period 3 did not differ significantly ($P > 0.01$) from that of any of the birds fed the 1.50 percent Ca diet during period 3. This suggests a carry-over effect of the diets fed during periods 1 and 2.

The results indicated that bone density indexes of layers fed 2.25 and 3.0 percent Ca diets in period 3 did not differ significantly ($P > 0.01$) regardless of the dietary Ca and P levels within a range of 2.25 to 3.0 percent Ca and 0.6 to 0.7 percent P, respectively, in periods 1 and 2. Similar results were also observed in trial 3, though in trial 3 the experimental diets were fed for 16, 12, or eight weeks.

The effect of the 1.50 percent Ca diet in lowering bone density index during period 3 was quite marked and was not dependent upon the level of Ca and P in the diet during periods 1 and 2. However, when diets containing 2.25 and 3.00 percent Ca were fed during periods 3 and 4, there tended to be a carry-over effect of the higher levels of Ca and P fed in periods 1 and 2.

The bone density index in period 4 followed the same pattern that was observed in period 3. No significant difference ($P > 0.05$) was observed in the bone densities of all layers fed a diet containing 1.5 percent Ca in this trial. At the 5 percent level of probability the bone density indexes of layers fed 2.25 percent Ca did not differ significantly with that of layers fed the same diet. At the 1 percent level of probability the bone density index of layers fed a diet containing 2.25 percent Ca in periods 3 and 4 did not differ significantly with that of layers fed 3.0 percent Ca diet in periods 3 and 4 regardless of the diets in periods 1 and 2. At the 5 percent level of probability the bone density indexes of layers fed a diet containing 3.0 percent Ca during periods 3 and 4 did not differ. As in period 3, the bone density indexes of layers fed the 2.25 percent Ca in periods 1 and 2 and the 2.25 or 3.0 percent Ca diets in periods 3 and 4 did not differ significantly ($P > 0.01$) from those of layers fed the 1.50 percent Ca diet in periods 3 and 4. This may be a carry-over effect of the diet fed during periods 1 and 2.

The results were interpreted to indicate that layers fed a low Ca diet (2.25 percent) may fail to respond the same as layers fed diets with higher levels of Ca.

The breaking strength of the tibiae is given in Table XXI. The breaking strength of tibiae of layers fed a diet containing 1.50 percent Ca did not differ significantly ($P > 0.05$) regardless of the diet fed in periods 1 and 2; it also did not differ with that of layers fed the 3.0 percent Ca diet in periods 1 and 2 and the 2.25 percent Ca diet in periods 3 and 4; or the 2.25 percent Ca in periods 1, 2, 3, and 4. The breaking strength of layers fed a diet containing 3.0 percent Ca in periods 3 and 4 did not differ significantly ($P > 0.10$) among themselves; and with that of the layers fed 2.75 percent Ca in periods 1 and 2 and 2.25 percent Ca in periods 3 and 4.

The different levels of Ca and P used in this trial failed to significantly affect breaking strength at the 5 percent level of probability. The variations of replicates in breaking strength were great. Similar variations were also found in the breaking strength of tibiae of layers in trial 3.

Incidence of Leg Weakness

The number of birds showing leg weakness in trials 1, 2, 3, and 4 is summarized in Tables XXII, XXIII, XXIV, and XXV, respectively. The word "sick" as used hereafter refers to those layers who showed leg weakness and/or cage layer fatigue (Avian osteoporosis).

Table XXI

Average Breaking Strength of Tibiae, Trial 4

Ca and P levels of diet				Average breaking strength kilograms
Periods 1 and 2		Periods 3 and 4		
Ca	P	Ca	P	
-----percent-----				
3.00	0.7	1.50	0.4	12.68 ^a
		2.25	0.6	20.68 ^{abcd}
		3.00	0.8	26.91 ^{cd}
2.75	0.6	1.50	0.4	13.57 ^a
		2.25	0.6	28.20 ^d
		3.00	0.8	23.49 ^{bcd}
2.25	0.6	1.50	0.4	16.09 ^{ab}
		2.25	0.6	18.46 ^{abc}
		3.00	0.8	22.53 ^{bcd}

¹Means with different small letter superscripts within the column differ significantly ($P \leq 0.10$) as determined by D. B. Duncan, 1955, Multiple range and multiple F test. Biometrics, 11:1-42.

Table XXII

Incidence of Leg Weakness in Layers Fed Diets with Ca:P
Ratios of 3.75:1, Trial 1

<u>Dietary Ca and P</u>		Number of birds with symptoms	Laying period in which symptoms occurred				
<u>Ca</u>	<u>P</u>		1	2	3	4	5
-----percent-----			-----number of birds-----				
1.50	0.4	14 ¹	10	3	5	1	0
2.25	0.6	8	5	1	2	0	0
3.00	0.8	3	1	1	1	0	0
3.75	1.0	2	0	1	0	1	0
4.50	1.2	2	0	2	0	0	0
Total		29	16	8	8	2	0

¹Five birds showed symptoms twice.

Table XXIII

Incidence of Leg Weakness in Layers Fed Diets with 3.00 and 3.50
Percent Ca and Varied Levels of P, Trial 2

Dietary Ca and P		Number of birds with symptoms	Laying period in which symptoms occurred				
Ca	P		1	2	3	4	5
-----percent-----			-----number of birds-----				
3.00	0.4	3	0	3	0	0	0
	0.6	3 ¹	2	1	1	0	0
	0.8	2	1	0	1	0	0
	1.0	0	0	0	0	0	0
	1.2	2	0	2	0	0	0
3.50	0.4	1	0	0	1	0	0
	0.6	3	0	3	0	0	0
	0.8	2 ¹	0	2	1	0	0
	1.0	0	0	0	0	0	0
	1.2	0	0	0	0	0	0
Total		16	3	11	4	0	0

¹One bird showed symptoms twice.

Table XXIV

Incidence of Leg Weakness in Layers Fed Diets of Varying
Levels of Ca and P, Trial 3

Ca and P level in diet						Number of birds with symptoms	Period in which symptoms occurred			
Period 1		Period 2		Periods 3 and 4			1	2	3	4
Ca	P	Ca	P	Ca	P		number of birds			
-----percent-----										
1.50	0.4	1.50	0.4	1.50	0.4	8	0	0	7	1
2.25	0.6	2.25	0.6	2.25	0.6	3	0	0	3	0
3.00	0.8	3.00	0.8	3.00	0.8	4	1	1	1	1
3.00	0.7	1.50	0.4	1.50	0.4	4	0	1	3	0
		2.25	0.6	2.25	0.6	2	0	0	1	1
		3.00	0.8	3.00	0.8	0	0	0	0	0
3.00	0.7	3.00	0.7	1.50	0.4	15	1	5	4	5
				2.25	0.6	3	2	1	0	0
				3.00	0.8	1	1	0	0	0
Total						40	5	8	19	8

Table XXV

Incidence of Leg Weakness in Layers Fed Diets of Varying
Levels of Ca and P, Trial 4

Periods 1 and 2				Periods 3 and 4			
Dietary Ca and P		Number of birds with symptoms		Dietary Ca and P		Number of birds with symptoms	
Ca	P	Period 1	Period 2	Ca	P	Period 3	Period 4
-----percent-----				-----percent-----			
3.00	0.7	0	5	1.50	0.4	0	5
				2.25	0.6	0	0
				3.00	0.8	0	0
2.75	0.6	1	0	1.50	0.4	2	0
				2.25	0.6	0	0
				3.00	0.8	0	0
2.25	0.6	3	1	1.50	0.4	2	0
				2.25	0.6	2	0
				3.00	0.8	0	0
Total		4	6			6	5

In trial 1 a total of 14, 8, 3, 2 and 2 birds fed the 1.50, 2.25, 3.00, 3.75, and 4.50 percent Ca diets, respectively, showed symptoms of leg weakness during the experiment. Five of the birds fed the 1.50 percent Ca diet showed symptoms twice. The number of birds with symptoms was 16, 8, 8, 2, and 0 during periods 1, 2, 3, 4, and 5, respectively.

In trial 2, no bird had leg weakness symptoms in periods 4 and 5 irrespective of dietary levels of Ca and P fed. There were no sick birds in the lots, which were fed the 3.00 or 3.50 percent Ca and 1.0 percent P diets or in those fed the 3.50 percent Ca, 1.2 percent P diet. The incidence of sick birds varied from one to three in the birds fed the other diets. If these sick birds are considered with respect to the P level in the diet, it appears that the number of sick birds was higher being 4, 6, and 4 in layers fed a diet containing 0.4, 0.6, and 0.8 percent P than those layers fed diet containing 1.0 and 1.2 percent P where the sick birds were 0 and 2, respectively.

In trial 3, when the layers were fed a diet containing 1.50 percent Ca for 16, 12, or eight weeks the number of sick birds was 8, 4, and 9, respectively. The number of sick birds decreased when the layers were fed a diet containing 2.25 percent Ca and 0.6 percent P for 16, 12, or eight weeks to 3, 2, and 0, respectively. When the layers were fed a diet containing 3.0 percent Ca and 0.8 percent P for 16, 12, or eight weeks the number of sick birds came to 4, 0, and 1, respectively. In this trial when the layers were fed a non-experimental diet containing 3.00 percent Ca for four or eight weeks, the number of sick birds was

4 during period 1 and 6 during period 2. It can also be noted that during periods 1 and 2 when the layers were fed a diet containing 3.0 percent Ca and 0.7 percent P, the number of sick birds was 10 out of 90.

In trial 4, there were 5, 1, and 4 sick birds during periods 1 and 2 when the layers were fed the 3.0, 2.75, and 2.25 percent Ca diets, respectively. In periods 3 and 4 there were 9, 2, and 0 birds sick when these layers were fed diets containing 1.50, 2.25 and 3.0 percent Ca, respectively.

During the four trials, 210 birds were fed a 1.5 percent Ca diet at some time during the experiment. Of this group 50 or 23.8 percent showed leg weakness at least once. Of the 270 birds fed the 2.25 percent Ca diet some time during these four trials, 19 or 7 percent showed leg weakness. During the four trials 750 birds were fed the 3.0 percent Ca diet at some time during the trials; of these 42 or 5.6 percent showed symptoms of leg weakness.

From these observations it appears that a larger number of sick birds can be expected when they are fed a diet containing 1.5 percent Ca and 0.4 percent P or 2.25 percent Ca and 0.6 percent P than when the layers are fed diets with either 3.0 or 3.50 percent Ca. In trials 3 and 4 there were instances where 3.0 percent Ca and 0.8 percent P seemed to prevent leg weakness but this did not hold true in trials 1 and 2.

None of the diets used completely prevented the occurrence of leg weakness.

Whenever a bird showed leg weakness symptoms she was X-rayed and whenever she appeared to be recovered she was again X-rayed. Feed was made available in a special cup to each sick bird. The X-ray of all sick birds could not be taken because some times the bone densitometer was inoperative due to an electronic malfunction, however, the bone densities of those sick birds determined when they went down and when they recovered are given in Table XXVI. These data are available on 21 incidences of leg weakness. It is interesting to note that out of 21 cases, 18 had a higher bone density index when they recovered than at the time they began to show symptoms. There were only two layers whose bone density index of tibia decreased during recovery, while the bone density of one bird did not change.

It was observed that these sick birds laid either to the day they became sick or they stopped laying about one or two days prior to showing symptoms. Many who became sick had laid soft shelled eggs. The sick birds did not resume laying until approximately a week after they had recovered. From these data and observations it appeared that when hens reached the critical point where they had to use too much of the reserve Ca from the bone to produce egg shell, they stopped laying.

Even though the birds were unable to stand, they did not appear droopy or have abnormal coloring around the head as is the case with many infectious diseases. Such birds ate freely from the cup containing feed provided in their cages and recovered. The condition of some birds deteriorated when the wing or leg broke or some other type of complication arose. In such condition they quit eating feed and died

Table XXVI

A Comparison of Bone Density Indexes Prior to the Occurrence of Leg Weakness Symptoms with Those at the Time Symptoms Occurred and After Recovery from the Symptoms

Dietary Ca and P CaP		Leg band number	Duration of sickness --days--	Bone density index		
				Prior to the symptoms	When symptoms appeared	After recovery
<u>Trial 1</u>						
1.5	0.4	7601	6	0.976	0.560	0.761
		7601	4	0.773	0.440	0.719
		7605	8	0.981	0.793	0.921
		7607	9	-----	0.606	0.739
		7844	3	1.093	0.653	0.810
		7845	3	1.018	0.715	0.748
		7850	3	-----	0.783	1.019
2.25	0.6	7620	3	1.009	0.732	0.886
		7625	14	-----	0.726	0.726
<u>Trial 2</u>						
3.00	0.8	7717	12	-----	0.698	0.703
		7937	13	-----	0.824	0.843
<u>Trial 3</u>						
1.50	0.4	4811	7	-----	0.756	0.809
		5042	15	0.862	0.851	0.871
		5051	7	-----	0.772	0.764
2.25	0.6	5108	5	-----	0.789	0.925
3.00	0.8	4840	4	-----	0.903	0.862
		5138	4	-----	0.778	0.913
		5142	11	-----	0.881	1.082
<u>Trial 4</u>						
1.5	0.4	4981	7	0.810	0.737	0.813
		4984	4	0.777	0.768	0.796
2.25	0.6	4993	4	-----	0.773	0.878

after some days. Those who recovered came to "normal" lay within a few days following recovery.

Variation in Response of Layers Fed the Same Diet as Measured
by Bone Density Index, Egg Production, Egg Specific
Gravity Scores, Bone Breaking Strength, and
Number of Times Birds Showed Symptoms
of Leg Weakness, Trial 1 and 2

Examples of variability in the response of birds to the criteria studied are given in Table XXVII. In trial 1, the bone density index of the tibia of one bird fed a diet containing 1.5 percent Ca and 0.4 percent P was 0.740, egg production for the entire experiment was 56 eggs, bone breaking strength was 6.4 kilograms, specific gravity score of her eggs was zero and she became sick twice. Another bird fed the same diet had a bone density index of 0.768, laid 102 eggs during the entire experiment, had a bone breaking strength of 9.5 kilograms, had an egg specific gravity score of eggs of zero, and she never became sick. These birds differed greatly in egg production and bone breaking strength. Another example of variability in this group was one bird that had a bone density index for the tibia of 0.950. She laid 45 eggs during the entire experiment, had a bone breaking strength of tibia of 14.0 kilograms, and was sick once. Another bird had a bone density of 0.846, she laid 93 eggs during the experiment, laid eggs with a specific gravity score of four and had a bone breaking strength of 14.9 kilograms.

Table XXVII

Examples of Variation in the Response of Layers Fed the Same Diet as Measured by Bone Density Index, Egg Production, Egg Specific Gravity Scores, Bone Breaking Strength, and Number of Times Bird Showed Symptoms of Leg Weakness, Trial 1 and 2

Dietary Ca and P		Bone density index	Eggs laid in		Eggs laid in last 28 days	Egg specific gravity score, last period	Bone breaking strength kilograms	Number of times bird had eeg weakness
Ca	P		141 days	number				
-----percent-----								
<u>Trial 1</u>								
1.50	0.4	0.740	56	9	0	6.4	2	
		0.768	102	20	0	9.5	0	
		0.950	45	1	-	14.0	1	
		0.846	93	21	4	14.9	0	
3.00	0.8	1.094	130	24	3	29.1	0	
		0.868	124	25	5	14.4	0	
		1.227	119	25	7	32.5	0	
3.75	1.0	1.306	114	20	2	76.4	0	
		1.203	114	24	5	10.7	0	
<u>Trial 2</u>								
3.00	0.4	1.210	17	5	-	47.7	0	
		0.897	121	25	5	15.0	0	
3.00	0.8	1.127	73	24	1	20.3	0	
		0.983	136	28	4	17.7	0	
3.50	1.2	1.093	126	21	0	36.9	0	
		0.894	137	27	4	18.3	0	

When the layers were fed a 3.0 percent Ca and 0.8 percent P diet, one bird had a bone density index of 1.094, laid 130 eggs during the experiment, laid eggs with a specific gravity score of 3.0 and had a bone breaking strength of 29.1 kilograms. Another bird on the same diet had a 0.868 bone density index, laid 124 eggs during the experiment, had a five egg specific gravity score and a 14.4 kilograms bone breaking strength. A third bird fed this diet had good bone density index (1.227), good egg production (119 eggs), good egg specific gravity score (7), and 32.5 kilograms bone breaking strength. These three layers had good egg production, good shell quality though it ranged from three to seven in specific gravity score, but one bird had poorer bone breaking strength (14.4 kilograms), and bone density (0.868) than the other two. This bird would most likely be considered satisfactory by a breeder, who did not measure bone density or bone breaking strength both of which could be considered relatively weak for birds fed this diet.

The bone density indexes and egg production in two layers fed 3.75 percent Ca and 1.0 percent P were very good but one bird had an egg specific gravity score of two and a bone breaking strength of 76.4 kilograms, while the other bird had an egg specific gravity score of five but a bone breaking strength of only 10.7 kilograms. A breeder selecting for shell quality would probably select the one which had a score of five but if he selects for bone strength, he would reject this bird because she had only a 10.7 kilograms breaking strength.

In trial 2, a bird fed 3.0 percent Ca and 0.4 percent P had a 1.210 bone density index, laid only 17 eggs during the experiment, but she had good bone breaking strength, 47.7 kilograms. This bird had satisfactory bone quality but not satisfactory egg production. Another bird fed the same diet had a bone density index of 0.897, laid 121 eggs, had an egg specific gravity score of five, and had a bone breaking strength of 15.0 kilograms. This bird would be suitable for egg production and for egg shell quality but not good for bone quality.

When the P level in above diet was raised to 0.8 percent, one bird was found to have a bone density index of 1.127, laid 73 eggs during the entire experiment, had a specific gravity score of 1 and had a bone breaking strength 20.3 kilograms. This bird would not be acceptable for egg production and shell quality. Another bird fed the same diet had a bone density index 0.983, she laid 136 eggs in the duration of the experiment, had an egg specific gravity score of four and had a bone breaking strength of 17.7 kilograms. This bird is good for laying and egg shell quality. When the layers were fed a 3.5 percent Ca and 1.2 percent P diet, a layer had a bone density of 1.093, laid 126 eggs, had an egg specific gravity score of 0, and had a bone breaking strength of 36.9 kilograms. This bird is not suitable for good egg shell quality. Another bird fed this diet laid 137 eggs had a bone density index of 0.894 an egg specific gravity score of four and bone breaking strength was 18.3 kilograms. This hen should be acceptable to the breeders.

The variation in the relationship in egg production, egg specific gravity score, bone density index, and bone breaking strength has been

previously discussed. There were variations in the criteria measured among layers within a lot fed the same diet under similar experimental conditions. Some layers had very good bone density indexes such as 1.094, 1.306, 1.127, and 1.093 and bone breaking strength 29.1, 76.4, 20.3, and 36.9 kilograms, respectively, but the egg specific gravity scores of these layers were 3, 2, 1, and 0. This indicates that when the bone density index and breaking strength are good it does not necessarily follow that the egg shell quality of these layers will also be good. There were layers which had 1.210, 1.227, and 0.897 values for bone density index, and 47.7, 32.5, and 15.0 kilograms of bone breaking strength and they laid 17, 119, and 121 eggs each, respectively, during the entire experimental period. It seems that egg production cannot be used as an indication of the bone density of a layer.

Some eggs had a high specific gravity score of 5, 7, 5, and 5. The respective layers' bone density indexes were 0.868, 1.227, 1.203 and 0.897 and the respective bone breaking strengths were 14.4, 32.5, 10.7, and 15.0 kilograms. It may be said that from egg specific gravity score the bone density index or tibiae breaking strength cannot be estimated.

The above described variations in the measurements obtained from layers for the criteria studied indicate that some birds when fed Ca deficient diets have the ability to lay more eggs, maintain better bone mineral levels and lay eggs with better shell quality than other birds fed the same diet. It can also be noted that some layers fed diets with adequate levels of Ca failed to lay eggs with high shell quality

and/or maintain high bone density scores or have high bone breaking strength. Such inconsistencies in response suggest definite genetic difference between individual birds. Primary poultry breeders can select birds on their ability to lay large numbers of eggs with strong shells but it does not follow that such birds will have bones with high bone density indexes or high breaking strength. While high bone density early in the laying period cannot be used to select layers that will not have symptoms of leg weakness, it does appear that birds that have the ability to lay a large number of eggs with strong shells and to maintain high bone density should be the most desirable birds for use in cage operations. Thus, it is suggested that breeders should begin selecting for such birds.

V. SUMMARY

A total of four trials involving 990 S. C. White Leghorn-type pullets were conducted to study the response of caged layers to various Ca and P levels as measured by bone density index, bone breaking strength, egg production, and egg specific gravity. An X-ray bone densitometer was used to determine bone density index and an Allo-Krammer shear press to determine the breaking strength of tibia.

One trial was designed to study the effects of graded levels of Ca and P in laying diets when the G:P ratios were maintained at 3.75 to 1. The diets were calculated to contain 1.5, 2.25, 3.0, 3.75 and 4.5 percent Ca. A second trial was designed to study the effects of two levels, 3.0 and 3.5 percent, of Ca with 0.4, 0.6, 0.8, 1.0 and 1.2 percent P. A third experiment was designed to study the effects of length of time laying pullets were fed a 3 percent Ca, 0.7 percent P diet on their response to diets containing 1.5 percent Ca and 0.4 percent P, 2.25 percent Ca and 0.6 percent P, and 3.0 percent Ca and 0.8 percent P. In a fourth trial, the layers were fed a diet containing 3.0, 2.75, and 2.25 percent Ca and 0.7, 0.6, and 0.6 percent P, respectively. This trial was conducted to study the effects of Ca level in the diet of laying hens on their response to higher and lower Ca and P levels in diets fed after the pullets were 29 weeks of age.

The results of trial 1 suggested that the requirement of Ca for maximum egg production may be less than 3.00 percent of the diet since the egg production of birds fed the 2.25 and 3.00 percent Ca diets did not differ significantly. The 1.50 percent Ca diet supported about 55 percent production during the experiment. During periods 3, 4, and 5 the egg production of these birds tended to plateau near 50 percent while birds fed the other diets continued to lay at about 80 percent.

The low level of Ca and P (1.50 percent Ca, and 0.4 percent P) reduced egg shell quality. The specific gravity of eggs from layers fed 3.75, and 4.50 percent Ca did not differ ($P > 0.05$).

The bone density indexes of layers fed diets with 1.5 percent Ca and 0.4 percent P were significantly lower ($P \leq 0.05$ and 0.01) than those of layers fed either 2.25, 3.00, 3.75 or 4.50 percent of Ca diet. However, at the 1 percent level of probability there was no difference in bone density when birds were fed diets with 2.25, 3.00, and 4.50 percent Ca or with 3.75 and 4.50 percent Ca. During the experimental period these bone densities tended to plateau.

The mean breaking strength of tibiae increased as the level of dietary Ca and P increased up to 3.75 percent Ca in the diet, but at the 5 percent level of probability no difference was observed.

In trial 2, layers fed, 3.0 and 3.5 percent Ca diets laid at the highest rate when the P content of the diet was 0.6 percent. However, the highest egg shell quality was obtained from birds fed a diet containing 0.4 percent P and the most suitable level of P for bone density was about 0.8 percent. The layers had higher bone density before the

experiment started, but when they were fed the experimental diets and were in peak of lay, they tended to maintain a certain almost constant level of bone density. It also appeared that the experimental diets had more influence on bone density in the beginning part of the lay and as the laying period increased this variability became less.

No significant difference in breaking strength of bones was observed when the layers were fed diets containing 3.0 or 3.5 percent Ca and 0.4, 0.6, 0.8, 1.0 or 1.2 percent P.

In trial 3, the diet containing 1.5 percent Ca and 0.4 percent P supported 50 percent egg production during period 4. This is comparable to the results obtained in trial 1.

The egg specific gravity score of layers fed a diet containing 1.5 percent Ca and 0.4 percent P for 16, 12, or eight weeks was significantly lower ($P \leq 0.05$) than with that of birds fed higher levels of Ca and P. The egg specific gravity score in the fourth experimental period was not affected when the higher dietary level of Ca and P (2.25 percent Ca, 0.6 percent P, and 3.0 percent Ca, 0.8 percent P) even though these diets had been fed for 16, 12, or eight weeks.

Between periods 3 and 4 a general decrease in egg specific gravity was found regardless of the diet fed. This may be because period 3 occurred in April when the environment was moderately warm and period 4 occurred in May when the temperature was warmer. The lowering in egg specific gravity score during the period of higher temperature might have been aggravated by the age of the layers. When the layers were

transferred from higher to lower level of dietary Ca and P the egg specific gravity dropped quickly.

When this diet containing 3.0 percent Ca and 0.7 percent P was fed and the layers were transferred to lower levels of Ca and P, this change of the diet quickly decreased bone density indexes. The duration of feeding 1.5 percent Ca, 0.4 percent P; 2.25 percent Ca, 0.6 percent P; and 3.0 percent Ca and 0.8 percent P diets had no influence on the bone density indexes even though the layers had been fed for 16, 12, or eight weeks.

No significant difference ($P > 0.05$) in breaking strength of the tibiae of layers fed any experimental diet in this trial for 16, 12, or eight weeks was found. However, there was a trend for the higher levels of dietary Ca and P to increase breaking strength. From these variations it may be concluded that diets fed to these layers before the experimental diets had no influence on the breaking strength of tibiae.

In trial 4 the egg production of layers fed a level of 1.50 percent Ca and 0.4 percent P was lower than that of layers fed either 2.25 or 3.0 percent Ca and 0.6 or 0.8 percent P, respectively, regardless of the previous treatment. The egg production of layers which had been fed a diet containing 3.0 percent Ca and 0.7 percent P during periods 1 and 2 and then transferred to a diet containing 1.50 percent Ca and 0.4 percent P was not as adversely affected in period 3 as were layers fed the same diet in periods 3 and 4 but fed either 2.75 or 2.25 percent Ca diets with 0.6 and 0.4 percent P, respectively,

in periods 1 and 2. It appears that there may have been a slight carry over effect of the higher level of dietary Ca fed in periods 1 and 2 into period 3 for layers fed the lower levels of Ca during period 3.

The egg shell quality probably was affected by the change of environmental temperature. The fall in the egg specific gravity score followed the same pattern as in trial 3.

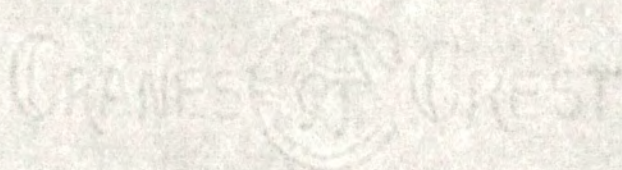
The results of the bone density determinations indicated that feeding layers a diet containing 2.75 or 3.00 percent Ca during periods 1 and 2 had no influence on the bone density indexes at the end of period 4 when the birds were fed diets containing 1.50, 2.25, and 3.0 percent Ca during periods 3 and 4. Any possible effects the diets fed during periods 1 and 2 may have had on bone density indexes in period 3 appears to have been lost by the end of period 4.

Layers fed a 2.25 percent Ca diet during period 1 and 2 and 2.25 or 3.00 percent Ca diets during periods 3 and 4 failed to respond as well during periods 3 and 4 and did birds fed diets with the higher levels of Ca during periods 1 and 2. This suggested a possible carry-over effect of the low Ca diet.

The different levels of Ca and P used in this trial failed to significantly affect breaking strength at the 5 percent level of probability.

Birds with symptoms of leg weakness (cage layer osteoporosis) were found in lots fed all diets. The highest incidence of this condition was in those birds fed the 1.5 percent Ca diet.

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