FACTORS INFLUENCING CALCIUM REQUIREMENT OF LAYERS
AS RELATED TO CERTAIN REPRODUCTIVE CHARACTERS

by

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1962

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Date thesis is presented _2-26-62_

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ACKNOWLEDGMENTS

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INTRODUCTION

The requirements of layers for calcium are greater than other farm animals. Laying birds need especially large amounts of calcium because egg shells are composed almost entirely of calcium carbonate. Calcium requirements for satisfactory egg production and egg shell quality have been studied for several years. However, in view of the number of changes that have occurred in the poultry industry in the succeeding years, the problem of adequate egg shell quality is still of practical importance to the poultryman.

The monetary loss which occurs from cracked or broken eggs has been estimated by the Ralston Purina Company in 1957 (68, p. 3) to be as high as 100 million dollars annually; thus it is a serious problem from an economic viewpoint. Poor egg shell quality is of practical importance in the poultry industry because in the process of transporting, handling, storage and incubation of eggs there are many occasions for breakage. Eggs with adequate shell quality have a much better chance to insure maximum returns to the producer.

The information on factors that might influence egg shell quality has been investigated during the past several years. Many of these factors are of nutritional significance. In

many instances the calcium requirement in past years has been met by free choice feeding of oyster shell in addition to the limited amount of calcium in the mash. It has also been assumed that layers will adjust their calcium requirements to meet production needs. Modern production rates are higher with present day rations and breeds. Furthermore many strains of birds now being used are smaller but at the same time producing eggs of at least equal size.

Continuing research on improving shell quality is the responsibility of investigators, feed manufacturers and producers in order to provide information on factors that might improve this problem and extend knowledge in this area. It is hoped this investigation will contribute in this direction.

REVIEW OF LITERATURE

Gross Nutrient Requirements

In order to produce eggs economically, a high rate of egg production accompanied by adequate egg shell quality must be maintained in a flock of laying hens. To secure this the hen must be fed a ration which is complete and well balanced and which meets fully the bird's nutritive requirements. Laying hens need a much greater content of calcium in their ration since the egg shell is composed almost entirely of calcium salts primarily calcium carbonate. Phosphorus, vitamin D_3 and manganese are also interrelated in egg shell formation. Thus, an adequate amount of phosphorus must be given to insure good shell formation. In addition, vitamin D_3 is also important in the metabolism of calcium and phosphorus and must be supplied at the proper level.

The 1960 revision of Nutrient Requirements of Poultry from the National Research Council (60, p. 21) recommends 2.25 percent calcium in rations for layers and breeders. The requirements of phosphorus and vitamin D_3 for laying hens are 0.6 percent and 225 I.C.U. per pound of feed. These recommendations are based on the results of several investigations which will be reviewed later.

Environmental Considerations

It is generally known that high environmental temperatures influence egg shell thickness. This fact was first reported by Bennion and Warren (6, p. 69-82). Since then numerous studies, including those of Miller and Bearse (58, p. 5-20) and Warren and Schnepel (91, p. 67-72) have shown that high environmental temperature results in the production of eggs with thin shells. These workers have demonstrated that temperatures above 70° F. are detrimental to shell quality by decreasing shell thickness rapidly. On the other hand, shell thickness is noted to improve after a subsequent decrease in temperature. These investigators also observed that high humidity accentuated the effect of high temperature, and that feed consumption of the hen at 95° F. was reduced 26 percent from that obtained at 60° F. Moreover, Warren and Schnepel concluded that the calcium content of the blood was also reduced in about the same proportion as was shell thickness when the birds were subjected to high temperatures. These results agree with those of Conrad (24, p. 328-329) who reported that increasing temperature from 70° to 90° F. caused a 25 to 35 percent decrease in the calcium level in the blood. In view of this the effect of temperature on egg shell thickness was attributed directly to the decreased calcium carrying capacity of the blood.

More recently, Wilson (93, p. 590-592) has shown that a

change in temperature from 70° to 100° F. results in a rapid

decrease in shell thickness. The hens lost weight progressively

as the ambient temperature was increased. Feed consumption at

100° F. was only 42 percent as compared to that at 70° F., while

water consumption increased to 135 percent. Egg production and

egg weight declined markedly as the ambient temperature in-

creased.

Petersen et al. (65, p. 21-22) reported that environmental

temperature exerted a marked effect on certain production

characters. In his initial experiments the laying house tem-

peratures were raised to 70-75° F. This was followed by an

immediate and sharp drop in egg shell quality as measured by

specific gravity. During a subsequent experiment, two tempera-

ture levels, 55° and 75° F., were studied. Shell quality was

significantly superior for eggs produced under the low tempera-

ture regime. The high temperature treatment resulted in approxi-

mately 8 percent less feed consumed, slightly lower egg weight

and body weight gains. They concluded therefore that tempera-

tures above 70° F. exert a detrimental effect on egg shell

quality.

Physiological Considerations

Romanoff and Romanoff (70, p. 157-158) noted that certain physiological considerations have been recognized as important factors in egg shell thickness. The efficiency of an individual hen's shell-secreting glands apparently varies. Often, as a result, the shell material is not deposited uniformly over the surface of the egg. That shell thickness usually changes throughout the laying cycle has been investigated. Romanoff (69, p. 351-356) reported that the strength of the shell tends to be less variable during periods of high egg production, when the secretory glands of the oviduct are apparently functioning in a more efficient manner. He obtained evidence showing differences in shell breaking strength between birds but, on the other hand, showed eggs laid by the same individual had more or less similar breaking strength characteristics. Blaskett, Dryden and Hale (14, p. 141-142) also found that the individuality of the hen was the most important factor in determining the breaking strength of the egg shell produced, although no significant differences between breeds were found.

Warren and Scott (90, p. 195-207) have shown that the time that the egg remains in the uterus affected shell formation. They also reported that thin-shelled eggs are due to the slower rate of calcareous-shell deposition.

The rate of egg shell formation has been studied by Burmester et al. (17, p. 101). This investigation found that egg shell deposition is relatively slow during the first three hours when the egg is in the uterus, and that after the third hour the rate of deposition increases rapidly and assumes a constant figure which is maintained until the time of laying.

Taylor and Lerner (80, p. 395-396) showed that the second egg of a clutch has a thicker shell than the first egg and that it was possible to eliminate the difference in shell thickness of the two eggs by selective breeding. Shell thickness has been observed to decrease during the laying cycle in an investigation by Wilhelm (92, p. 248-249). In addition when observations were made on eggs per clutch evidence was obtained that the shell of the second egg in 2 egg cycles was thicker than that of the first eggs and in cycles of 3 or more eggs, the shells of the first and last eggs were the thickest. Those of the intervening eggs were progressively thinner, except when the cycle consisted of five or more eggs. The next to the last egg then bears a shell some what thicker than that of the egg immediately preceding it.

Berg (7, p. 562-563) reported that the eggs with the thickest shell are laid after the longest interval of time. It appears that the relative thickness of the shell depends to some extent upon the time available for the accumulation of shell-forming substances. He also indicated that the shell of

the second egg of the two egg clutches was thicker than that of the first egg. In clutches of three or more eggs the first and last eggs were thicker than those of the intervening eggs.

Age. Wilhelm (92, p. 250) investigated certain factors affecting variation in egg shell quality. He reported that the relationship between percentage of shells and egg shell thickness was highly correlated and not independent of egg weight. He also observed that with mature egg size both shell thickness and dry weight of the shell decline while egg size remains relatively constant. Halloran and Maxwell (40, p. 15-16) have shown that the percentage of shell and its thickness decline from the first pullets' eggs through the second year of production. Additional investigations by Pfost (67, p. 56) also showed a decline of egg shell quality as affected by the age of the hens from six to twenty-nine months. He reported that the average egg shell thickness ranged from 0.0143 to 0.0132 inches. The change in shell thickness appears to decrease with increasing age of the hen. Egg shell thickness from six to twelve months was greater than from twelve to twenty-nine months. Bernier (11, p. 23) reported that the level of shell strength progressively decreases as the laying year progresses but the differences of genetic origin between families and individual hens remain relatively constant throughout the year.

Strain. Taylor and Lerner (80, p. 383-396) have presented evidence that egg shell quality is inherited. They demonstrated this by establishing two strains of chickens each laying preponderantly thin-shelled and thick-shelled eggs. Romanoff and Romanoff (70, p. 153) stated that the thickness of the individual hen's egg shell is a manifestation of her calcium metabolism. The efficiency in assimilating and secreting calcium and other minerals involved in shell formation apparently comes under hereditary control to some extent. Variation in average shell thickness from one flock to another may be due in part to accentuation of family differences through selective breeding.

Strain and Johnson (73, p. 46) have investigated the seasonal, hatchability and strain effects on egg quality. They obtained results from three different strains of two breeds and have shown that the specific gravity and hatchability are different between strains in the same breed. Moreover, Petersen et al. (66, p. 1283) reported a large difference in shell quality between strains beginning with the third week of the experiment. Shell quality of the low-shell-quality strain when fed 3.75 percent calcium was lower than obtained with the high-shell-quality strain fed 2.25 percent calcium.

Body Weight. Bernier and Arscott (12, p. 1234-1235) reported that dwarf hens weighed 63 percent as much, had a proportionately smaller skeleton and laid eggs with thinner shells which

may indicate more critical requirements for calcium, phosphorus and/or vitamin D.

Endocrinological Considerations

Romanoff and Romanoff (70, p. 245) noted that the mobilization of calcium and phosphorus involves a complex interplay of hormonal effects. The resting level of calcium in the blood is directly controlled by the secretion of the parathyroid glands which are controlled by the pituitary gland. As pituitary activity increases in the female bird during reproduction, the parathyroids are stimulated to greater activity, and blood calcium and phosphorus levels are elevated. Sun and MacOwan (76, p. 4-5) found that the microscopical structure of the parathyroid glands in the laying hen differed from that of the non-laying hen. In the former the cells were larger, not as closely packed and frequently exhibited a degree of ovulation. MacOwan (55, p. 384) reaffirmed her previous work on the structure of the parathyroid gland during the period of ovulation by injecting chickens with parathormone. The samples of blood were taken seventeen hours after injection. She was able to show an increased calcium level in the serum of pullets.

Knowles et al. (51, p. 84,85,87) reported that an increase in the blood calcium of immature pullets and non-laying hens with 1 to 3 cc. of parathormone, but they got no response in cocks or capons. However, they concluded that the period of low

level of calcium in the blood corresponds to the time taken for shell deposition. The period of high level corresponds to the absence of a fully formed egg in the shell gland. Ash (1, p. 2) obtained results by using pooled data from three strains of chicken. A highly significant negative correlation was found to exist between the number of cells per unit volume of parathyroid tissue and the percentage of shell. As the number of parathyroid cells increased, the specific gravity of eggs laid decreased. A possible interpretation of this correlation is that the larger parathyroid cells are secreting more parathormone which brings about the mobilization of greater amounts of diffusible calcium for the production of egg shells.

Sturkie (74, p. 386) noted that most of the positive results with parathormone were obtained in laying females, in which presumably the ovaries are secreting estrogen. This suggests that parathormone and estrogen have synergistic effects and that the latter may play a major role in the mobilization of calcium. Flieschmann and Fried (31, p. 409) reported that estrogen increases calcium in the blood of immature chicken in presence of intact parathyroids.

Asmundson and Pinsky (2, p. 103-104) reported shell weight and thickness of eggs from hens fed desiccated thyroid to be significantly greater than control birds. Gabuten and Shaffner (33, p. 49-53) showed that thyroxin and estrogen tended to increase specific gravity of chicken eggs while progesterone and

thiouracil decreased egg shell thickness. Gutteridge and
Novikoff (39, p. 212) reported that, following thyroprotein
treatment, there was a highly significant increase in egg shell
strength, as measured by specific gravity of the eggs.

Hoffmann and Wheeler (49, p. 610-611) obtained positive
results by feeding thyroprotein to pullets. They reported that
there was a highly significant increase in specific gravity of
the eggs in the treated pullets. Wilson (93, p. 591) added
iodized casein to the ration of layers and reported an increase
in shell thickness. This was confirmed by Berg and Bearse (10,
p. 21-28).

Gutowska and Parkhurst (38, p. 203) reported that the phos-
phatase activity in the blood plasma of laying hens is related
to their productivity. The physiological mechanism for the
deposition of calcium in the egg shell is independent on a local
phosphatase activity factor in the shell gland of the hen. They
also suggested that the transformation of the colloid compound,
containing calcium and phosphorus and yielding calcium for the
egg shell, takes place in the blood itself with the shell gland
acting as an excretory organ of calcium only. Gutowska and
Mitchell (36, p. 167) proposed that carbonic anhydrase acts as
a catalyst in the shell gland for the decomposition of carbonic
acid, thus allowing carbonate ions to be formed from bicarbonate
ions. These carbonate ions are then utilized in calcium car-
bonate deposition with calcium being derived by the shell gland

from calcium proteinate which is present in the blood.

Mehring et al. (57, p. 1388) reported that feeding 50-100 mg. of sulfonamide or 35 mg. of benzensulfonamide daily caused the pullets to lay eggs with very thin shells or with no shell at all, but after the treatment was stopped a few days the birds again laid eggs with normal shells. They also indicated that sulfonamide and benzensulfonamide inhibit the action of carbonic anhydrase and, hence, the secretion of shell material. This observation agrees with the conclusions reached by Benesch et al. (5, p. 138-139) and Burnard and Genest (16, p. 617-618) who, working with sulfanilamide, found it to be an effective inhibitor of the secretion of egg shell material. These workers also reported that other sulfonamides such as sulfathiazole, sulfaquanidine, and sulfadiazine have no noticeable inhibitory effect on shell secretion.

Pathological Considerations

It is not the purpose of this review to deal extensively with pathological aspects of egg shell thickness. However to show that such a problem may exist the following reports are cited.

Carson et al. (19, p. 594-595) reported that chronic respiratory disease affects hens by decreasing egg production and egg shell thickness. The antibiotic injected birds were able to maintain shell thickness as well as egg production.

Egg weight and albumen quality were not improved by the treatment. In this respect infectious bronchitis and infectious coryza also have been reported to affect egg production and egg shell thickness as reported by Van Roekel et al. (89, p. 146), and Taylor et al. (79, p. 132-133), respectively.

Lorenz and Newlon (53, p. 197-198) observed that pneumoencephalitis caused abnormal egg quality as manifested by bubbly eggs, decreased albumen height, and abnormal shell. Berg et al. (9, p. 617, 619, 622) reported that Newcastle disease decreased egg shell thickness from 0.347 mm. to 0.316 mm. or 8.9 percent. This disease also affected the smoothness of the egg shells by increasing the roughness of the shells. Egg production decreased markedly and a loss in albumen quality was reported. Clegg and Mueller (21, p. 158) reported that Newcastle disease affects egg shell thickness by the direct effect of the disease on the uterus or upon the disturbance of factors controlling shell secretion.

Nutritional Considerations

Romanoff and Romanoff (70, p. 154) noted that shell thickness is influenced by the amount of several nutrients contained in the bird's diet, especially calcium, phosphorus, vitamin D and probably manganese. However, the capacity of the hen to utilize these elements for shell deposition is extremely variable and depends upon individual differences in the physiological

processes governing assimilation, secretion and excretion. Sturkie (74, p. 274) also noted that the shell is composed of approximately 94 percent calcium carbonate, 1 percent magnesium carbonate, 1 percent calcium phosphate and 3-4 percent of organic matter, chiefly protein.

Driggers and Comar (26, p. 423-424) reported that after feeding radioactive calcium (Ca^{45}) to laying hens the activity of radioactive calcium was found in the shell of an egg which was laid 15 minutes after administration. It was estimated that from 60-75 percent of the calcium in an egg is secured directly from the diet. O'Niel et al. (62, p. 778-779) investigated the uptake of calcium in eggs by feeding radioactive Ca^{45} to a hen. They obtained results showing that the majority of the labeled calcium was located in the shell. The maximum recovery was within 43 hours after feeding.

Calcium Levels. Norris et al. (61, p. 308-309) have investigated the calcium requirement of laying hens and concluded that 1.65-1.8 percent of calcium is just sufficient to meet the requirement as judged by egg production, shell strength and egg shell ash. In relating phosphorus to the calcium requirement they reported that 0.75 percent phosphorus was adequate for this purpose. Titus et al. (85, p. 126-127) reported the calcium requirement for White Leghorn pullets was 2.2 percent when the level of phosphorus content was 0.9 percent. A high

level of calcium intake adversely affected the hatchability of
the eggs.

Evans and Carver (27, p. 469) indicated that the best pro-
duction was obtained with the pullets receiving 2.5 percent
calcium, 0.8 percent phosphorus and 60 A.O.A.C. chick units of
vitamin D per 100 grams of diet. When these levels were lowered
a decrease in egg shell thickness and egg production occurred.
Gutowska and Parkhurst (37, p. 327-328) reported the results of
feeding hens all mash rations containing different levels of
calcium with normal phosphorus and ample vitamin D. The calcium
requirement of laying hens under these conditions was reported
as 2.0 percent with 3.95 percent detrimental to egg production
and feed utilization.

Evans et al. (29, p. 39, 41) reported that calcium at a
level of 3.0 percent and 0.8-1 percent phosphorus gave the best
results when egg shell thickness was used as the criterion. A
level of 2.5 percent calcium allowed satisfactory production as
did 3.0 percent. Petersen et al. (65, p. 22-25) reported that
calcium levels of 2.25 plus free-choice whole oyster shell as
well as 3.00, 3.38, 3.75, 4.50 and 5.25 percent calcium in the
ration resulted in improved shell quality when compared to 2.25
percent in the basal ration. Maximum shell quality appeared to
be obtained with 3.75 percent calcium when the birds were kept
at environmental temperatures of 50°-60° F. When calcium levels
of 2.25, 3.75, 4.50 and 5.25 percent were used with a house

temperature of 75° F., calcium intake of 4.50 and 5.25 percent was required for optimum shell quality. None of the calcium levels studied had a detrimental effect upon egg production. Calcium levels of 4.50 and 5.25 percent did result in increased feed consumption and pounds of feed required to produce a dozen eggs. Body weight gains during the laying year were also slightly less than when the higher calcium levels were fed. They concluded that the level of 2.25 percent calcium was not adequate for production of eggs with maximum shell quality.

The National Research Council (60, p. 21) has established the calcium requirement for laying and breeding hens at 2.25 percent, phosphorus at 0.6 percent and vitamin D_3 at 225 I.C.U. per pound of feed. They also indicated that this amount of calcium need not be incorporated in the mixed feed, inasmuch as calcium supplements fed free choice may also be considered as part of the ration. These recommendations are based on the reports of Norris et al. (61, p. 309), Evans and Carver (27, p. 469), Miller and Bearse (58, p. 5-20) and Murphy et al. (59, p. 4-10).

Helbacka (43, p. 46-50) reported that eggs laid by hen receiving 3.75 percent calcium carbonate had significantly thicker shells than did shells of the lower treatments. It was demonstrated that hens fed a ration containing 2.25 percent calcium compared to 3.75 percent calcium laid three times as many eggs of inferior shell quality as the latter during the end of the

laying season. The number of eggs falling in this category was much greater at the beginning of the season. Additional studies were undertaken later to determine the effects of calcium and phosphorus on shell quality by using 2.25, 3.0, 3.75 and 4.50 percent of calcium levels with 0.5 and 0.7 percent phosphorus. The results with calcium levels were the same as in the previously reported study. The higher phosphorus level resulted in thicker shells although the breaking strength pattern appears somewhat different. It was concluded that raising the calcium level of the hen's ration above the National Research Council's recommendation improved shell quality with no detrimental effects on production or any of the egg quality factors studied.

Tyler and Wilcox (88, p. 60-61) found that hens which consumed 0.5 gram of calcium per day retained about 70 percent, whereas only 50 percent was retained at an intake level of 2.0 grams per day. The latter intake would amount to 1.0 gram retention which is to be the maximum amount that could be retained. Common (23, p. 219-220) has reported that with high producing hens, up to 1.0 gram calcium intake daily resulted in about 70 percent retention and that with 1.0 to 3.5 grams calcium intake per day the retention remained at about 50 percent. He also concluded that the optimum calcium requirement for laying hens was about 4.0 grams per day.

Petersen et al. (65, p. 22) calculated the calcium retention from their experiment based upon data of Common and

reported that with calcium levels of 2.25, 3.75, 4.50 and 5.25 percent the daily calcium retained was 1.27, 2.21, 2.65 and 3.20 grams, respectively. Since an average size egg contains approximately 2.0 grams of calcium, the daily calcium retained would be 1.27 grams or only about 60 percent of the amount required for shell formation with 2.25 percent calcium in the diet.

Dietary Antibiotics. Gabuten and Shaffner (33, p. 50-52) reported that procaine penicillin at the level of 30 ppm in the ration improved shell thickness as measured by specific gravity of the eggs. As the season progressed, the eggs from the controls continued to decline in specific gravity but the treated birds did not show this decline. When the treatment was stopped, the specific gravity of the eggs from the treated birds declined and within two weeks approached that of the eggs from the control. In the second trial they fed procaine penicillin at 15 and 30 ppm in the ration during high environment temperatures. They observed a noticeable decline in the specific gravity of eggs from the control group. The birds receiving 15 ppm penicillin laid eggs which declined slightly in specific gravity from 1.081 to 1.0807 while the control group laid eggs that declined in specific gravity from 1.0804 to 1.073. The group given 30 ppm not only maintained the pre-treatment level but the specific gravity of their eggs increased slightly. They also reported that the penicillin feeding increased blood

calcium with the increase related to the dosage.

Bogdonoff and Shaffner (15, p. 1044) studied the effect of antibiotics on calcium metabolism of laying hens to determine their effect on plasma calcium in blood and specific gravity of eggs. These results showed that penicillin feeding increased blood plasma calcium and specific gravity of the eggs in the treated group although blood plasma calcium levels were highly variable. Cadet (18, p. 60) reported that the addition of 10 to 40 ppm of antibiotics, such as bacitracin, penicillin and terramycin to the laying ration during the summer months gave a positive and favorable effect upon egg shell thickness as measured by specific gravity of the eggs. This improved egg shell thickness was accompanied by the maintenance of higher serum calcium levels in the blood of layers receiving supplementary antibiotics.

Ross and Yacowitz (71, p. 263-265) reported highly significant increases in bone ash of chickens receiving penicillin. They indicated that penicillin enhances bone calcification and increases calcium absorption but the increased absorption is probably mediated through vitamin D since no increase in bone ash was noted in the absence of or at a very low level of vitamin D.

Several investigators have indicated that antibiotic effects on egg shell thickness seem to be insignificant. To illustrate, the following reviews are cited.

Chin and Brant (20, p. 875-876) found that aureomycin at levels of 5, 10, 20 and 40 ppm in the diet did not improve shell thickness as determined with a micrometer or egg weight. Bearse and Berg (4, p. 1180) reported no significant differences in egg shell quality, albumen quality or in blood spot incidence between eggs of birds fed 0, 10, and 100 grams of chlortetracycline per ton of feed. The antibiotic did, however, cause a significant increase in yolk color.

Heywang and Vavich (48, p. 1001-1002) fed White Leghorns rations containing 0, 50, 100 and 200 grams of chlortetracycline per ton to determine the effect of antibiotic feeding during hot weather on egg weight and shell quality. They reported that under conditions of their experiment no appreciable change in egg weight or shell quality was observed for any of the levels of antibiotic supplementation.

Heywang and Kemmerer (47, p. 1034-1035), who used the ratio of dried-shell weight to whole-egg weight as the measure of shell quality, found no appreciable change in shell quality or egg weight during hot weather when procaine penicillin was fed at the level of 30 ppm of the diet. In the experiment of Petersen et al. (64, p. 798) procaine penicillin at the levels of 4, 20 and 100 grams per ton of diet did not improve the specific gravity or size of eggs from White Leghorn pullets either before or after the introduction of high environmental temperature.

Dietary Ascorbic Acid. Thornton and Moreng (83, p. 595,597, 598) reported that 10 mg. of ascorbic acid per pound of feed containing 2.303 percent calcium and 0.716 percent phosphorus increased egg shell thickness with environmental temperatures of 70° and 82° F. Eggs from the ascorbic acid supplemented hens had shells which were thicker to a highly significant degree under both environmental conditions. Shell thickness for both groups was reduced when the hens were subjected to the higher temperature; however, this reduction was much greater for the controls than the treated hens. Shell strength also declined more in the control hens under the warm environmental conditions as did body weight of these hens.

The relationship of vitamin C to calcium metabolism has been reported by Thornton et al. (84, p. 37-38) who noted that the presence of vitamin C in the parental diet affected Ca^{45} uptake and retention markedly in control and rachitic progeny. In these studies it was observed that rachitic chicks from vitamin C supplemented parents were able to take up and deposit Ca^{45} in the skeleton at a rate comparable to control chicks. Rachitic chicks from control parents were unable to do this. It was proposed this observation was due to the fact that the carry-over of parental ascorbic acid enhanced intestinal absorption of Ca^{45} in the absence of vitamin D, improved bone deposition per se, or both factors were involved. It was also noted that the loss of Ca^{45} from the skeleton of

rachitic progeny from vitamin C supplemented parents was accelerated when compared to rachitic progeny from control parents. This latter evidence suggested that the increased Ca^{45} uptake and loss from the skeleton was due to a more rapid turnover rate in this tissue in progeny from the vitamin C supplemented parents.

The effect of dietary calcium level on the efficiency of ascorbic acid was investigated by Thornton (82, p. 1402-1405) who reported that the addition of 20 mg. of ascorbic acid per pound of diet containing 2.0 percent calcium appeared to have no influence on the shell thickness. At the 2.5 and 3.0 percent level of calcium the ascorbic acid supplement was highly beneficial for maintenance of shell quality during the summer months. He indicated that the birds given the ascorbic acid at the low calcium level absorbed less calcium; thus had less of this mineral to use for egg shell formation resulting in the thinner shells. He concluded that dietary ascorbic acid was effective in maintaining shell thickness during periods of increased environmental temperatures when a sufficient calcium level was used. He also indicated that the calcium requirement for egg production may be increased when vitamin C was added to the diet.

Pepper et al. (63, p. 1283) reported that 15 mg. of ascorbic acid per pound of feed containing 1.0, 2.5, and 4.0 percent calcium had no effect on shell quality during

temperatures of 75-80° F. Ascorbic acid tended to decrease blood calcium at the high levels of calcium while the results were inconsistent at the lowest level of calcium. They concluded that the ascorbic acid effect was not significant. Helbacka (43, p. 47) reported that at normal temperatures vitamin C failed to have a marked effect on shell thickness in rations containing 2.25 percent calcium.

Bearse (3, p. 4) reported that in a diet containing 2.5 percent calcium with and without free choice oyster shell, supplementary ascorbic acid at 20 grams per ton of feed did not result in an improvement in shell quality as determined by specific gravity and shell smoothness.

Sources of Calcium. Ewing (30, p. 526) noted that the most common sources of calcium that have been used in feeding poultry are limestone and oyster shell. They appear to be of equal value insofar as results are concerned, provided, of course, the limestone is of good quality and not from dolomitic sources. Massengle and Platt (56, p. 244-245) reported that birds receiving chemically pure calcium carbonate as a source of calcium did not grow as well, lay as many eggs or produced eggs with as thick shells as those receiving oyster shell or limestone. They also indicated that calcium in the form of oyster shell or limestone is utilized better than calcium in phosphate and that birds receiving limestone seem to have a slightly better shell

texture than those receiving oyster shell.

Heuser and Norris (45, p. 173-179) found that crushed oyster shell and calcite grit fed as supplements to a laying ration gave better results than when ground limestone was included in the mash. Egg shell strength was rated in descending order as follows: oyster shell, calcite grit and ground limestone. Body weights appeared to be maintained better in the case of hens receiving crushed oyster shell than those receiving calcite grit or gound limestone. These workers suggested that the best combination was crushed oyster shell and granite grit. Russell and McDonald (72, p. 473-474) reported that laying pullets can utilize calcium from calcium citrate as well from calcium carbonate for egg formation. The breaking strength grade, egg weight and percentage of calcium in the shell were equal in these respects for both sources of calcium. Berg, et al. (8, p. 20-21) reported that equally good results in egg production and egg shell quality were obtained whether the calcium was supplied in the mash or as limestone grit.

Heywang (46, p. 221-222) involving two experiments at the Arizona Agricultural Experiment Station, compared calcium gluconate, technical-grade calcium lactate, calcium d-lactate, precipitated calcium carbonate and calcium sulphate with the calcium carbonate of high-grade limestone as sources of egg shell calcium for White Leghorn hens. No differences were noted on the ratio of dried-shell weight to whole-egg weight from the

hens receiving different sources of calcium during periods of either high or moderate air temperatures.

Other Nutrients. Evans et al. (28, p. 14-15) reported that layers fed a diet containing 2.5 percent calcium needed 0.8 percent phosphorus for best egg production during the first 4 months when egg shell thickness was used as the criterion. Six-tenths of one percent phosphorus gave poorer production than 0.8 percent when the diets contained 2.5 percent calcium. No significant differences were observed between 0.8, 1.0 and 1.2 percent phosphorus during the last 6 months of their experiment. Gillis et al. (35, p. 983-984) used diets containing 1.9 to 2.0 percent calcium and found that layers needed 0.6 percent of readily available phosphorus.

Crowley et al. (25, p. 33-37) fed a practical type basal diet containing 0.41 percent total phosphorus and supplemented with soft phosphate, dicalcium phosphate and a combination thereof to White Leghorn pullets. The control group received no supplemental phosphorus. They found that percentage of air-dried shell and shell phosphorus content declined in all lots as the study progressed. The decline was more rapid in birds fed no supplemental phosphorus than in hens receiving phosphorus supplementation.

Bird (13, p. 11-12) stated that phosphorus is just as essential as calcium for egg formation, but the results of

phosphorus deficiency are less specific and less spectacular than are the results of calcium deficiency. A deficiency of calcium leads to thin shells but a deficiency of phosphorus leads in most cases only to a lower rate of egg production.

Bearse (3, p. 3) mentioned that phosphorus levels in the diet do not seem to have an effect on egg shell quality, nevertheless, it should be pointed out that phosphorus is involved in the deposition and withdrawal of the skeletal calcium used in shell formation. However, he concluded that it is essential to have a minimum of 0.4 percent inorganic phosphorus in laying rations.

Ewing (30, p. 552) stated that supplying a necessary mineral for shell manufacture is not necessarily proof that eggs with adequate shells will be laid unless the hens also get plenty of vitamin D. Taylor and Martin (81, p. 44) reported that vitamin D is a factor influencing thickness of egg shells. With insufficient amounts of vitamin D, the hens will produce thin or soft-shelled eggs. Titus (86, p. 807) noted that a deficiency of vitamin D in the diet affects the thickness of the shells and their ability to resist breakage. Taylor (78, p. 11) stated that vitamin D is closely related to the assimilation and metabolism of calcium and phosphorus, and consequently a shortage of this vitamin results in thin-shelled eggs.

Lyons (54, p. 18) reported that pullets fed a deficient manganese ration with either high or low levels of calcium and

phosphorus produced eggs with distinctly different and inferior shell characteristics than those fed adequate amounts of manganese. When a number of pullets on the manganese-deficient diets were changed to manganese-adequate diets, a rapid and marked improvement in shell quality occurred, while pullets changed from adequate to deficient-manganese levels showed an even more rapid decrease in egg shell quality.

Bearse (3, p. 3) has stated that manganese is related to shell quality by functioning in egg shell formation primarily as a component of certain enzyme systems which are necessary for the proper absorption and utilization of calcium and phosphorus. The National Research Council (60, p. 21) suggests a level of 15 mg. manganese per pound of ration for breeding hens.

Supplee et al. (77, p. 1246) reported, in preliminary work with White Leghorn hens maintained in batteries on an isolated soy-protein diet, supplementary zinc improves hatchability of fertile eggs and slightly increases shell thickness. In a study with floor pens, supplemental zinc did not improve egg production or shell quality during 24 weeks of production.

Bearse (3, p. 3) noted that zinc may also be involved in shell formation because it is a component of the enzyme system, carbonic anhydrase, which is an important factor in the conversion of calcium from the feed to calcium in the egg shells. However, he concluded that addition of zinc to ordinary laying rations is of questionable value, apparently because sufficient

amounts are being supplied by the ingredients commonly used.

Helbacka and Hall (44, p. 1211) demonstrated that feeding of 2 percent ammonium chloride in the laying ration caused a decrease in shell thickness but significantly improved albumen quality.

EXPERIMENTAL PROCEDURE

Experiment I. Normal Size Layers Fed
Differing Calcium and Oleandomycin Levels

Ninety-six normal size Oregon Agricultural Experiment Station White Leghorn pullets were housed in individual cages in a forced draft ventilated room prior to the onset of production in September, 1959. All birds were raised throughout the growing period under a routine management program followed by the Department of Poultry Science.

The pullets were divided into triplicate lots of 8 birds each. The experimental treatments were initiated in October. The experimental outline is shown in Table VI (page 42) with differing calcium and oleandomycin levels serving as variables. The composition of experimental rations used is given in Table I. Feed and water were supplied *ad* *libitum* and at least 14 hours of light were provided throughout the experiment. Daily egg production, mortality, monthly feed consumption and initial and final body weights were recorded during ten 28-day periods.

Specific gravity and egg weight determinations were made on eggs laid during three successive days at the end of each 28-day period throughout the trial. Specific gravity was determined daily in aqueous salt solutions involving values of 1.044 to 1.104, with 0.004 intervals, using the procedure

TABLE I

Composition of Experimental Rations

Experiment I

FEEDSTUFFS	RATION (%)			
	1 (2.25%)[1]	2 (3.0%)	3 (2.25%)	4 (3.0%)
Corn, yellow, grd.	72.50	70.60	72.50	70.60
Soybean meal, solv. (44% prot.)	13.75	13.75	13.75	13.75
Fish meal (70% prot.)	3.00	3.00	3.00	3.00
Alfalfa meal, dehy. (20% prot.)	3.00	3.00	3.00	3.00
Fat, animal[2]	1.00	1.00	1.00	1.00
Limestone flour	4.50	6.40	4.50	6.40
Bone meal, steamed	1.25	1.25	1.25	1.25
Salt, iodized	0.50	0.50	0.50	0.50
Vit. and min. premix[3]	0.50	0.50	0.50	0.50
Oleandomycin (4 gms./T)	-	-	+	+
Total	100.0	100.0	100.0	100.0

1. Figures in ( ) represent calcium level.

2. Calogen, stabilized with Tenox R which is composed of 20% citric acid (anhydrous), 20% butylated hydroxyanisole and 60% propylene glycol.

3. Supplies per lb. of mixture: 500,000 U.S.P.U. Vit. A; 200,000 I.C.U. Vit. D_3; 100 I.U. Vit. E.; 0.35 gms. riboflavin; 2 gms. niacin; 0.3 gms. pantothenic acid; 20 gms. choline; 0.4 mgs. Vit. B_{12} act.; 11.34 gms. butylated hydroxytoluene; 5.4 gms. Mn; 0.109 gms. I; 1.8 gms. Fe; 0.182 gms. Cu.

described by Bernier (11, p. 16-23). Blood calcium and phosphorus determinations and body weights were obtained every third 28-day period throughout the trial. Blood calcium and phosphorus measurements were made by using the procedures adapted from Hawk et al. (42, p. 579, 589)[1]. The method of Fredrickson (32, p. 390-391) for obtaining blood from the brachial vein was employed.

During March and April all layers were inseminated by using pooled New Hampshire semen. Hatching eggs were obtained 2 days after insemination, collected for 7 days and incubated. There were four hatches obtained in this experiment. The day-old chicks from each hatch were sacrificed and stored in a freezer according to treatment until used.

Chicks obtained were dried to a constant weight in a drying oven at 100-104° C. for 72-96 hours. Ashing each chick was accomplished in crucibles at 600° C. for 30 minutes. Chicks from the last hatch were ashed at 600° C. for 2-1/2 hours.

Experiment II. Dwarf Layers Fed Differing Calcium Levels and Ascorbic Acid

The study involving the dwarf layers was conducted for two 28-day periods during June and July, 1960. These layers had

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1. Determinations were conducted in the Department of Agricultural Chemistry by Mr. Frank Adams.

previously completed a feeding trial (Exp. III) involving six-teen 28-day periods and were randomly allocated to individual cages prior to the onset of the trial. Each treatment con-sisted of four lots of four hens each. The rations used are given in Table II, and the experimental outline is shown in Table IX (page 47). Except for blood calcium and phosphorus data, the procedures used and information collected were similar to those for the normal size hens previously described.

Experiment III. Dwarf and Normal Size Layers Fed Differing Calcium-Phosphorus Levels

One hundred and twenty dwarf White Leghorn pullets and an equal number of their normal size sisters were randomly allo-cated to individual cages prior to the onset of production in September, 1959. All birds received at least 13 hours of light per day. Each treatment consisted of five lots of four hens each. Feed and water were supplied _ad_ _libitum_ with calcium-phosphorus serving as the major variable in all rations. In addition to receiving a 15 percent protein control ration with two calcium-phosphorus levels, a similar ration with double the vitamin-trace-mineral supplementation and an 18 percent protein ration with double the vitamin-trace-mineral supple-mentation were also fed. The experimental outline is shown in Table X (page 46). All rations were formulated to contain similar Calorie-protein ratios and are shown in Table III.

TABLE II

Composition of Experimental Rations

Experiment II

FEEDSTUFFS	RATION (%)		
	1 (2.25%)[1]	2 (2.25%)	3 (3.0%)
Corn, yellow, grd.	72.50	72.50	70.60
Soybean meal, solv. (44% prot.)	13.75	13.75	13.75
Fish meal (70% prot.)	3.00	3.00	3.00
Alfalfa meal, dehy. (20% prot.)	3.00	3.00	3.00
Fat, animal[2]	1.00	1.00	1.00
Limestone flour	4.50	4.50	6.40
Bone meal, steamed	1.25	1.25	1.25
Salt, iodized	0.50	0.50	0.50
Vit. and min. premix[3]	0.50	0.50	0.50
Ascorbic acid (20 gms./T)	-	+	-
Total	100.0	100.0	100.0

1. Figures in ( ) represent calcium level.

2. See footnote 2, Table I.

3. See footnote 3, Table I.

TABLE III

Composition of Experimental Rations

Experiment III

FEEDSTUFFS	RATION (%)					
	1 (2.25-0.6%)[1] '15% prot.'[2]	2 (3.0-0.9%) '15% prot.'	3 (2.25-0.6%) '15% prot.'	4 (3.0-0.9%) '15% prot.'	5 (2.25-0.6%) '18% prot.'	6 (3.0-0.9%) '18% prot.'
Corn, yellow, grd.	72.50	70.00	72.00	69.50	48.45	45.95
Soybean meal, solv.(44% prot.)	13.75	13.75	13.75	13.75	21.50	21.50
Fish meal (70% prot.)	3.00	3.00	3.00	3.00	3.00	3.00
Alfalfa meal, dehy. (20% prot.)	3.00	3.00	3.00	3.00	3.00	3.00
Fat, animal[3]	1.00	1.00	1.00	1.00	15.50	15.50
Limestone flour	4.50	4.50	4.50	4.50	4.50	4.50
Bone meal, steamed	1.25	3.75	1.25	3.75	1.25	3.75
Salt, iodized	0.50	0.50	0.50	0.50	0.50	0.50
Vit. and min. premix[4]	0.50[5]	0.50	1.00	1.00	1.00	1.00
Methionine hydroxy analogue	-	-	-	-	0.05	0.05
Zein	-	-	-	-	1.00	1.00
Choline chloride (25%)	-	-	-	-	0.25	0.25
Total	100.0	100.0	100.0	100.0	100.0	100.0

1. Figures in ( ) represent calcium-phosphorus level.
2. Figures in ' ' represent protein level.
3. See footnote 2, Table I.
4. See footnote 3, Table I.
5. Figure underlined represents level of vit. and min. premix used.

Egg production, feed consumption and body weight data for the treatments were collected during the following 28-day periods: 3rd-4th, 5th-8th, 11th-13th and 14th-16th. Calcium-phosphorus levels were switched within ration treatments beginning with the 5th and 14th periods. All birds received the same regimen during the 1st-2nd and 9th-10th periods. Specific gravity determinations were made daily on eggs laid during 4 successive days at the end of each 28-day period throughout the trial.

Experiment IV. Normal Size Layers Fed Differing Levels and Sources of Calcium

Seventy-two normal size Oregon Agricultural Experiment Station White Leghorn pullets were divided into triplicate lots of 8 birds each and housed in individual cages in a forced draft ventilation room prior to the onset of production in September, 1960. The study was conducted for ten 28-day periods from October, 1960 to July, 1961. The experimental outline is given in Table XIII (page 50). The experimental rations are shown in Table IV. Management, egg production, feed consumption, mortality, specific gravity and egg weight were made and recorded as described in Experiment I except that blood calcium and phosphorus determinations were not undertaken in this study.

During January and February the birds were inseminated with White Leghorn semen. Methods of collection and incubation were

TABLE IV

Composition of Experimental Rations

Experiment IV

FEEDSTUFFS	RATION (%)		
	1 (2.25%)[1]	2 (2.25%)	3 (3.25%)
Corn, yellow, grd.	73.68	73.13	71.60
Soybean meal, solv. (44% prot.)	13.37	13.27	13.00
Fish meal (70% prot.)	2.92	2.90	2.84
Alfalfa meal, dehy. (20% prot.)	2.92	2.90	2.84
Limestone flour	0.97	0.97	0.95
Oyster shell flour	2.73	2.37	5.47
Bone meal, steamed	2.43	2.41	2.36
Salt, iodized	0.49	0.48	0.47
Vit. and min. premix[2]	0.49	0.48	0.47
Calcium lactate (91.5%)	-	1.09	-
Total	100.0	100.0	100.0

1. Figures in ( ) represent calcium level.

2. See footnote 3, Table I.

the same as in Experiment I. There were four hatches obtained
in this experiment. Ashing of the right metatarsus was similar
to the ashing procedure described in Experiment I using a 48-
hour drying period and a 30-minute ashing time.

Experiment V. Normal Size Layers
Fed Differing Calcium Levels

Four hundred White Leghorn pullets of the Oregon Agricul-
tural Experiment Station strain were equally distributed into
eight 16' x 16' floor pens during July, 1960 when they were
approximately 5 months of age. The pens were equipped with
either constant flow or automatically regulated waterers as well
as adequate feeder and nesting space. At least 14 hours of
light were provided during the experiment.

Feed and water were supplied _ad_ _libitum_. The experimental
outline is shown in Table XV (page 53). The experimental ra-
tions are given in Table V. Daily egg production, mortality,
monthly feed consumption, initial and final body weights were
recorded. Specific gravity and egg weight determinations were
obtained during twelve 28-day periods in a manner similar to
that described in Experiment I. No culling was practiced dur-
ing the experiment.

Statistical Procedure

All data on specific gravity, egg production, blood calcium
and phosphorus values and embryo ash values for the normal size

TABLE V

Composition of Experimental Rations

Experiment V

FEEDSTUFFS	RATION (%)			
	1 (2.25%)[1]	2 (2.75%)	3 (3.25%)	4 (3.75%)
Corn, yellow, grd.	73.68	72.63	71.60	70.56
Soybean meal, solv. (44% prot.)	13.37	13.18	13.0	12.81
Fish meal (70% prot.)	2.92	2.88	2.84	2.79
Alfalfa meal, dehy. (20% prot.)	2.92	2.88	2.84	2.79
Oyster shell flour	2.73	4.11	5.47	6.85
Limestone flour	0.97	0.96	0.95	0.93
Bone meal, steamed	2.43	2.40	2.36	2.33
Salt, iodized	0.49	0.48	0.47	0.47
Vit. and min. premix[2]	0.49	0.48	0.47	0.47
Total	100.0	100.0	100.0	100.0

1. Figures in ( ) represent calcium level.

2. See footnote 3, Table I.

hens were statistically treated using analysis of variance and Least Significant Differences[1] computed as described by Li (52, p. 553). In Experiment V egg production, specific gravity and feed consumption data for the 2.75 percent treatment level during the ninth 28-day period were estimated because water intake was severely restricted due to a clogged water line that severely reduced the rate of lay. The dummy values used in these data were computed by the method of Li (52, p. 209).

Since no significant interaction was observed between treatment x period, a pooled error mean square[2] was obtained and utilized in calculating the L.S.D. values for the various data obtained. Statistics for day-old chicks in Hatches I, II and III were computed separately from Hatch IV because the ashing procedure for chicks in Hatch IV involved a longer period of time.

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1. L.S.D. $= \sqrt{\dfrac{2 \text{ (error mean square)}}{\text{number of observations per treatment}}}$

2. Pooled error mean square $= \dfrac{\text{sum (error SS for each treatment involved)}}{\text{sum (error d.f. for each experiment involved)}}$

RESULTS

Experiment I. Normal Size Layers Fed
Differing Calcium and Oleandomycin Levels

The summary of results on the influence of differing calcium and oleandomycin levels on specific gravity of eggs and certain other performance characteristics of normal size White Leghorn layers is given in Table VI. Specific gravity of eggs was significantly improved ($p < 0.01$) in the presence of 3.0 percent calcium or the combination of 3.0 percent calcium and oleandomycin. The addition of oleandomycin to the 2.25 percent calcium level also significantly increased specific gravity of eggs but to a lesser extent ($p < 0.05$). Specific gravity values for the hen receiving 3.0 percent calcium and oleandomycin were significantly higher than all other treatments ($p < 0.01$). A decline was noted in specific gravity of eggs for all treatments as the study progressed.

Egg production was not significantly affected by any of the dietary treatments. There were no consistent effects on egg weight, feed consumption or feed per dozen eggs for any treatment. No marked treatment effects on body weight or mortality were noted.

Blood calcium and phosphorus values are summarized in Table VII. No significant differences were obtained from the various treatments.

TABLE VI

Effect of Calcium (Ca) and Oleandomycin (Ol'n) Levels on Specific Gravity (Sp.gr.)
and Performance of Normal Size White Leghorn Layers

Treatment[1]	Sp. gr. ($\times 10^3$-1000)	Egg prod.[2,4] (%)	Egg wt.[5] (gms.)	Feed cons./[3,5] hen/period (lbs.)	Feed/ doz.[5] eggs (lbs.)	Gain body[5] wt. (lbs.)	Mortality[5] (%)
2.25% Ca	77.3	57.1	57.2	5.59	4.43	0.04	12.5
3.00% Ca	79.3	56.7	56.4	6.03	4.61	0.03	4.2
2.25% Ca + Ol'n (4 gms./T)	78.5	61.6	58.0	5.62	3.95	0.21	12.5
3.00% Ca + Ol'n (4 gms./T)	81.0	58.5	55.8	5.47	4.69	0.19	4.2
L.S.D. ($p < 0.01$)	1.4						
L.S.D. ($p < 0.05$)	1.1						

1. Average of triplicate lots (8 hens/lot) for ten 28-day periods.
2. Hen day basis.
3. Feed consumption calculated from actual count of 280 days.
4. No significant difference.
5. Not statistically treated.

TABLE VII

Effect of Differing Calcium (Ca) and Oleandomycin (Ol'n)
Levels on Blood Calcium and Phosphorus

Treatment[1]	Blood calcium[2] (mg./100 ml. plasma)	Blood phosphorus[2] (mg./100 ml. plasma)
2.25% Ca	23.96	4.40
3.00% Ca	23.61	4.20
2.25% Ca + Ol'n (4 gms./T)	24.31	4.68
3.00% Ca + Ol'n (4 gms./T)	23.11	4.21

1. See footnote 1, Table VI.
2. No significant difference.

The results from ashing day-old chicks are summarized in
Table VIII. Highly significant differences $(p < 0.01)$ in percent
ash were obtained from day-old chicks from hens fed 3.0 percent
calcium with or without oleandomycin during Hatches I, II and
III. It is also of interest to note that the combination of
3.0 percent calcium and oleandomycin resulted in significantly
greater percent ash than the 3.0 percent calcium level alone
$(p < 0.01)$. For Hatch IV only the combined 3.0 percent calcium
level and oleandomycin resulted in a highly significant differ-
ence $(p < 0.01)$.

Experiment II. Dwarf Layers Fed
Differing Calcium Levels and Ascorbic Acid

The results for dwarf layers fed differing calcium and
ascorbic acid levels are summarized in Table IX. The addition

Table VIII

Influence of Dietary Calcium (Ca) and Oleandomycin (Ol'n)
on Ashing Day-old Chicks

Treatment	% Ash[1]	% Ash[2]
2.25% Ca	13.11 (162)[3]	7.32 (23)[3]
3.00% Ca	13.55 (180)	7.19 (40)
2.25% Ca + Ol'n (4 gms./T)	13.33 (157)	7.24 (26)
3.00% Ca + Ol'n (4 gms./T)	14.28 (131)	8.32 (25)
L.S.D. (p < 0.01)	0.32	0.81

1. Average from Hatches I, II and III.
2. Average from Hatch IV.
3. Figures in ( ) represent no. chicks ashed.

of ascorbic acid to the ration resulted in no improvement on
specific gravity of the eggs. On the other hand, the dwarf
layers fed the 3.0 percent calcium level produced eggs which
gave significantly higher specific gravity values than all other
treatments (p < 0.05).

No significant differences in egg production and feed con-
sumption were obtained for any dietary treatment. There were
no consistent effects on egg weight for any treatment.

Experiment III. Dwarf and Normal Size Layers
Fed Differing Calcium-Phosphorus Levels

The summary of results on specific gravity and performance
of normal and dwarf White Leghorn layers fed varying calcium-
phosphorus levels is given in Tables X and XI. Specific gravity

TABLE IX

Effect of Ascorbic Acid (Vit. C) and Calcium (Ca) Levels
on Specific Gravity (Sp.gr.) and Performance of Dwarf White Leghorn Layers

Treatment[1]		Sp. gr.	Egg production[2]	Egg weight[3]	Feed consumption[2]
Ca(%)	Vit. C (mg./lb.)	$(X10^3-1000)$	(%)	(gms.)	(lbs.)
2.25	0	68.4	37.1	57.2	4.35
2.25	10	68.9	33.8	57.6	4.30
3.00	0	73.4	41.4	57.6	4.12
L.S.D. $(p < 0.05)$		4.0			

1. Average of duplicate lots (4 hens/lot) for two 28-day periods.
2. No significant difference.
3. Not statistically treated.

TABLE X

Effect of Calcium-Phosphorus (Ca-P) Levels on Specific Gravity (Sp.gr.)
and Performance of Normal and Dwarf White Leghorn Layers

Treatment[1]		Sp. gr.	Egg prod.	Egg wt.	Feed cons.	Feed/ doz. eggs	Body weight	
Ca(%)	P(%)	$(X10^3-1000)$	(egg/bird)	(gms.)	(lbs./bird)	(lbs.)	Gain (lbs.)	Final (lbs.)
				NORMAL				
2.25	0.6	78.7	13.7	60.5	6.15	5.39	0.13	4.51
3.00	0.9	80.3	14.4	61.0	6.40	5.33	0.25	4.55
				DWARF				
2.25	0.6	73.8	11.2	53.9	3.97	4.25	0.08	2.89
3.00	0.9	77.0	12.9	54.6	4.30	4.00	0.18	2.95

1. Average 15 lots (4 hens/lot) for twelve, 28-day periods.

TABLE XI

Effect of Protein Level and a Vitamin-Trace-Mineral Supplement (DVTMS)
on Specific Gravity (Sp.gr.) and Performance of Normal and Dwarf White Leghorn Layers

Treatment[1]	Sp. gr. $(X10^3-1000)$	Egg prod. (egg/bird)	Egg wt. (gms.)	Feed cons. (lbs./bird)	Body weight	
					Gain (lbs.)	Final (lbs.)
NORMAL						
15% prot.	79.2	14.4	61.2	6.58	0.15	4.66
15% prot. + DVTMS	80.4	14.2	61.0	6.73	0.18	4.49
18% prot. + DVTMS	79.0	13.6	60.5	5.53	0.24	4.62
DWARF						
15% prot.	75.0	11.7	54.6	4.22	0.10	2.88
15% prot. + DVTMS	76.8	12.3	54.3	4.47	0.12	2.95
18% prot. + DVTMS	74.5	12.2	53.9	3.70	0.18	2.94

1. See footnote 1, Table X.

values of eggs were improved by the high calcium-phosphorus
level but more so for the dwarfs than the normals. Values for
the dwarfs receiving the high mineral level still were below
those of the normals receiving the low mineral level. Doubling
the vitamin-trace-mineral supplement in the 15 percent protein
ration resulted in improving egg shell thickness. No beneficial
effect was noted in the ration containing 18 percent protein with
double the vitamin-trace-mineral supplementation. These results
indicated that a greater need exists for a nutrient(s) contained
in a vitamin-trace-mineral supplement.

Egg production was improved approximately 15 percent for
the dwarfs and 5 percent for the normals fed the higher calcium-
phosphorus level. However, the dwarfs receiving the higher
mineral level still laid 6 percent fewer eggs than the normals
receiving the lower mineral level. A slight increase in pro-
duction was noted for the dwarfs fed the 15 percent protein diet
and double the vitamin-trace-mineral supplement. No improvement
was observed when the protein level was increased to 18 percent.

Egg weight was not affected by any dietary treatment with
normal or dwarf layers. However, the normal hens laid eggs that
were about 10 percent heavier than the dwarfs. Feed consumption
was increased approximately 4 percent for the normals and 8 per-
cent for the dwarfs fed the high calcium and phosphorus level.
Even so the dwarfs fed the high calcium level consumed about 30
percent less feed than the normals receiving the low level.

The results for blood calcium and phosphorus levels are summarized in Table XII. The blood of normal and dwarf hens receiving the high calcium-phosphorus level appear to contain slightly more blood calcium and phosphorus. However, in view of the variability encountered between replicates it is doubtful these observations are meaningful.

TABLE XII

Effect of Calcium-Phosphorus (Ca-P) Level on
Blood Calcium and Phosphorus Content of Normal
and Dwarf White Leghorn Layers

	Treatment[1]		Blood Ca	Blood P
	Ca (%)	P (%)	(mg./100 ml. blood)	(mg./100 ml. plasma)
Normal	2.25	0.6	20.99	4.04
	3.00	0.9	21.34	4.43
Dwarf	2.25	0.6	20.38	3.99
	3.00	0.9	21.07	4.05

1. See footnote 1, Table X.

Experiment IV. Normal Size Layers Fed
Differing Levels and Sources of Calcium

The results involving calcium carbonate and calcium lactate and differing calcium levels on specific gravity and performance of normal size White Leghorn layers are given in Table XIII. The addition of 1 percent calcium lactate to the ration resulted in no improvement on specific gravity of eggs. On the

TABLE XIII

Effect of Calcium Sources and Calcium (Ca) Levels on Specific Gravity (Sp.gr.)
and Performance of Normal Size White Leghorn Layers

Treatment[1]	Sp. gr. $(X10^3 - 1000)$	Egg prod.[2] (%)	Egg wt.[3] (gms.)	Feed cons./ hen/period[3] (lbs.)	Feed/ doz. eggs[3] (lbs.)	Gain body wt.[3] (lbs.)	Mortality[3] (%)
2.25% Ca	78.3	69.66	58.5	6.89	4.56	0.85	12.50
2.25% Ca (1% Ca-lactate)	78.8	67.31	56.8	6.46	4.30	0.70	4.20
3.25% Ca	80.5	73.04	57.3	6.42	3.82	0.92	8.30
L.S.D. $(p < 0.01)$	1.5	4.2					

1. Average of triplicate lots (8 hens/lot) for ten 28-day periods.
2. Hen day basis.
3. Not statistically treated.

other hand, egg shell thickness was significantly (p < 0.01) in-
creased with 3.25 percent calcium.

No significant difference was observed for egg production
between calcium levels. However, the hens fed the high calcium
level produced significantly more eggs than hens fed 2.25 per-
cent calcium with 1 percent calcium lactate (p < 0.01). In view
of the fact that no significant difference exists due to source
when comparison is made within the same calcium level, the
validity of the statistical interpretation is subject to
question.

Feed consumption, feed per dozen eggs, body weight gains
and mortality appeared not to be greatly influenced by any of
the dietary treatment.

The results of ashing the metatarsus are summarized in
Table XIV. No significant difference from ashing the metatar-
sus due to the various dietary treatments were noted.

TABLE XIV

Influence of Dietary Calcium Sources and Calcium (Ca)
Levels on the Ash of the Metatarsus in the Day-old Chick

Treatment[1]	% Ash
2.25% Ca	13.77 (174)[2]
2.25% Ca (1% Ca-lactate)	14.06 (255)
3.25% Ca	14.29 (253)

1. Average from four hatches.
2. Figures in ( ) represent number of metatarsus ashed.

Experiment V. Normal Size Layers
Fed Differing Calcium Levels

The results regarding calcium levels on specific gravity
and performance of normal size White Leghorn layers are summar-
ized in Table XV. Egg shell thickness of hens fed the three
higher calcium levels was improved significantly ($p < 0.01$) over
the control diet. In addition, the specific gravity of eggs
produced by hens receiving 3.75 percent calcium appeared signifi-
cantly greater than the 2.75 percent calcium level at the 5
percent level of probability. However, the 3.25 percent level
did not prove significantly different from either the 2.75 or
3.75 percent levels.

Egg production of the hens fed 3.25 percent calcium level
was found to be significantly greater than all other treatments
($p < 0.01$). There were no significant differences in egg pro-
duction between the control and the remaining treatments.

No differences for egg weight, feed consumption, body
weight and mortality attributable to the various dietary treat-
ments were observed.

TABLE XV

Effect of Calcium (Ca) Levels on Specific Gravity (Sp.gr.)
and Performance of Normal Size White Leghorn Layers

Treatment[1]	Sp. gr. (X10^3-1000)	Egg prod.[2] (%)	Egg wt.[3] (gms.)	Feed cons./[3] hen/period (lbs.)	Feed/ doz.[3] eggs (lbs.)	Gain body wt.[3] (lbs.)	Mortality[3] (%)
2.25% Ca	78.13	68.80	56.3	6.35	4.16	0.86	1.1
2.75% Ca	79.13	67.82	55.4	6.48	4.21	0.77	2.4
3.25% Ca	79.25	74.06	55.7	6.75	3.94	0.73	1.1
3.75% Ca	79.63	68.65	54.8	6.66	4.21	0.71	2.3
L.S.D. (p<0.01)	0.63	2.26					
(p<0.05)	0.50						

1. Average of duplicate lots (50 hens/lot) for twelve 28-day periods.
2. Hen day basis.
3. Not statistically treated.

DISCUSSION

The results obtained from numerous experiments indicate
that the 2.25 percent of calcium cited by the National Research
Council (60, p. 21) for laying hens is inadequate for the main-
tenance of good egg shell thickness. For the experiment in-
volving various calcium levels (Table XIV) a level of 2.75 per-
cent is indicated as adequate in the laying ration for optimum
egg shell thickness since no significant differences occur be-
tween 2.75-3.75 percent at the one percent level of probability.
Although a significant difference may be noted between the 2.75
and 3.75 percent levels at the five percent level of probability
this is considered of minor importance since no differences are
apparent under similar conditions between 2.75 and 3.25 or 3.25
and 3.75 percent.

A method for predicting the calcium requirement of laying
hens by considering the calcium required to produce a dozen
eggs has been reported by Combs and Helbacka (22, p. 32). They
estimated that approximately 50-60 percent of the calcium was
excreted in the droppings and about 40-50 percent calcium used
for egg formation. They further noted that a dozen two-ounce
eggs contained about 24 grams of calcium; thus, the laying hen
requires approximately 54 grams (0.12 pounds) of feed calcium
to produce a dozen eggs. From this, they presented an easy
method to predict calcium required in the ration by dividing

0.12 pounds of calcium in a dozen eggs by the feed consumed per dozen eggs and multiplying the quotient by 100 to give the percentage of calcium needed in the ration. Based on this method one can estimate the calcium level needed in the control groups of Experiments I, IV and V (Tables VI, VIII and XIV) utilizing actual data on feed required per dozen eggs as 4.43, 4.56 and 4.16 pounds, respectively. Predicted calcium levels obtained from this method were 2.71, 2.63 and 2.88 percent for an average of 2.77 percent in contrast to the level of 2.25 percent employed. On the basis of the higher calcium levels employed in Experiments I, IV and V (Tables VI, VIII and XIV) where 2.75, 3.0, 3.25 and 3.75 percent calcium was used and 4.21, 4.61, 3.82-3.94 and 4.21 pounds of feed required per dozen eggs, the predicted calcium levels in these rations should be 2.85, 2.60, 3.14-3.05 and 2.85 percent which provides an average of 2.91 percent.

It would appear, therefore, from these calculations that required calcium levels may range betwen 2.77 and 2.91 percent. For practical purposes 3.0 percent calcium may be recommended that would allow some margin of safety. These results are in general agreement with the reports of Titus (87, p. 10), Petersen et al. (65, p. 25), Helbacka (43, p. 46-50) and Harms (41, p. 34, 36) who also indicate that the level of 2.25 percent calcium is not adequate for the production of eggs with increased shell thickness. In general, however, these reports

indicate that levels above 3.75 percent are required for maximum shell thickness. It is unfortunate that these investigators for the most part failed to use calcium levels between 2.25 and 3.75 percent. Recently, Sullivan and Kingan (75, p. 96) have reported that 2.8 percent calcium was needed by high producing hens in order to produce maximum egg shell thickness.

The results obtained from the dwarfs and normal size sisters in Experiment III (Table X) with differing calcium-phosphorus levels indicate that the dwarf layers fed 3.0 percent calcium laid eggs with thinner shells as well as about 6 percent fewer eggs than their normal sisters fed 2.25 percent calcium. Increasing the calcium-phosphorus level from 2.25-0.6 percent to 3.0-0.9 percent resulted in improving egg shell thickness. The improvement was more pronounced in the dwarfs than their normal sisters although shell thickness for the dwarfs was still not comparable to that of the normals. In view of this one can postulate that the dwarfs may require more calcium to meet physiological needs than do normal layers. Based on the method of Combs and Helbacka (22, p. 32-33) one can predict the calcium level needed using actual feed required per dozen eggs as 5.33-5.39 pound for the normals and 4.00-4.25 pound for the dwarfs. Predicted calcium levels obtained were 2.25-2.23 and 3.00-2.82 percent, respectively. These values suggest that the dwarf layers require higher calcium levels than the normal sisters. Bernier and Arscott (12, p. 1234-1235) reported that the dwarf

hens had a proportionately smaller skeleton, that they laid eggs with less shells and were more susceptible to cage fatigue which may be taken to indicate more critical requirements for calcium, phosphorus and/or vitamin D.

The improvement of egg shell thickness was noted in the ration containing 15 percent protein with doubling the vitamin-trace-mineral supplement. This may point out that a greater need exists for nutrient(s) contained in a vitamin-trace-mineral supplement. Taylor (78, p. 11) stated that vitamin D is closely related to the assimilation and metabolism of calcium and phosphorus. Furthermore, Bearse (3, p. 3) mentioned that manganese is related to shell thickness through involvement in certain enzyme systems and through proper absorption and utilization of calcium and phosphorus. The beneficial effect from doubling the vitamin-trace-mineral supplement may therefore be due to increased vitamin D and/or manganese content in the ration.

No beneficial effect was noted in the ration containing 18 percent protein with double the vitamin-trace-mineral supplement. It should be mentioned that the 18 percent protein ration may have failed to improve egg shell thickness because the layers consumed less feed. This may have been due to the fact that this ration contained the same Calorie-protein ratio as did the 15 percent protein diet through the addition of 15.5 percent fat to the diet, thus markedly increasing the energy content of the diet. Since it had been established that the hen regulates

its food intake on the basis of the energy content of the diet as reported by Combs and Helbacka (22, p. 32-33), the decreased feed consumption noted above may have resulted in decreased nutrient intake.

The results obtained in Experiment I (Table VI) indicate that feeding oleandomycin at 4 grams per ton appeared to improve egg shell thickness to a lesser extent with 2.25 percent calcium as compared to hens receiving 3.0 percent calcium level. It is possible that oleandomycin may increase calcium digestion and utilization which results in improving egg shell thickness. That antibiotics affect egg shell thickness has been reported by Gabuten and Shaffner (33, p. 50-52), Bogdonoff and Shaffner (15, p. 1044) and Cadet (18, p. 1-60) who have indicated that penicillin, terramycin and bacitracin feeding increased egg shell thickness as measured by specific gravity. This improved egg shell quality was accompanied by the maintenance of higher serum calcium levels in layers fed supplementary antibiotics.

It must be also noted that some reports in the literature have failed to show beneficial effects on shell thickness resulting from antibiotic administration. Chin and Brant (20, p. 875-876), Bearse and Berg (4, p. 1180), Heywang and Vavich (48, p. 1001-1002), Heywang and Kemmerer (47, p. 1034-1035) and Petersen et al. (64, p. 798) have indicated that antibiotics were not effective in improving egg shell thickness.

The results of blood calcium-phosphorus analysis in Experiment I (Table VII) indicate that addition of oleando-mycin and 3.0 percent calcium singly or in combination to the hen's diet did not show significant differences even though egg shell thickness was improved. These blood calcium-phosphorus data are not in agreement with those results reported above from the Maryland Station (15) (33) and McGill University (18) which mentioned that antibiotic feeding increased blood plasma calcium, but do agree with the results of Heywang and Vavich (48, p. 1001-1002) who indicated that antibiotics did not effect the blood plasma calcium of the laying hens.

The data obtained in Experiment III (Table IX) indicate that addition of 10 milligrams per pound of ascorbic acid to the dwarfs' ration was without effect in improving egg shell thickness as measured by specific gravity. It should also be noted that this trial was conducted in cages during June and July, 1960, under higher than average environmental tempera-tures and with birds that have been shown to be more sensitive to calcium levels than normal sized layers. These results are not in agreement with the results reported by Thornton et al. of the Colorado Station (83, p. 595), (84, p. 38) but do agree with results reported by Pepper et al. (63, p. 1283), Helbacka (43, p. 47) and Bearse (3, p. 4) who failed to obtain improve-ments in egg shell thickness by addition of ascorbic acid to the ration of layers.

The results obtained in Experiment IV (Table XIII) indicate that calcium lactate in laying rations did not increase or decrease egg shell thickness as measured by specific gravity. It should be noted that the calcium lactate groups contained approximately 1 percent calcium lactate and 3.34 percent calcium carbonate. These results are in agreement with reports by Helbacka (43, p. 46-47) who indicated that eggs laid by hens receiving calcium lactate were similar in shell thickness to eggs laid by hens receiving calcium carbonate when levels of 2.25 and 3.0 percent calcium were used. The breaking strength data for shells was also in good agreement with data for shell thickness. Heywang (46, p. 218) also reported that a good source of calcium in laying rations is calcium carbonate from limestone. Calcium lactate alone or a combination of calcium lactate and calcium carbonate as sources of egg shell calcium were no better than calcium carbonate alone.

The data for ashing day-old chicks in Experiment I (Table VIII) are of interest. The three percent calcium level resulted in a significant difference on ash content of day-old chicks in Hatches I, II, and III but not IV. Highly significant increases in ash content of day-old chicks were obtained from hens fed 3.0 percent calcium with oleandomycin for all hatches. It would appear from these data that more complete ashing eliminated the response with the higher calcium level.

In view of this one may propose that the combination of oleandomycin and the high calcium level in the diet not only increases egg shell thickness but also may increase the internal calcium content in the yolk of eggs so the embryo from groups receiving the combinations might obtain more calcium than low calcium groups. While the above view is certainly a possibility, additional data should be obtained to confirm and extend this observation.

The results from ashing metatarsus of day-old chicks in Experiment IV (Table XIV) indicate that there were no significant differences between calcium levels or calcium sources. The 3.0 percent calcium level resulted in slightly higher ash content in the metatarsus but this was not of sufficient magnitude to be of significance.

In support of the above observations, Ross and Yacowitz (71, p. 263-265) have indicated that penicillin enhances bone calcification and increases calcium absorption in rations containing sufficient vitamin D. They obtained highly significant increases in bone ash from chicks receiving penicillin in the ration. Taylor (78, p. 189) found that egg shells provide the largest part of calcium which is deposited in the embryo. The calcium content of the embryo increases when heavy ossification of the embryonic skeleton begins.

None of the calcium levels studied had a detrimental effect upon egg production for normal size hens. The results obtained

in Experiments I, II and IV (Tables VI, IX and XIII) indicate
that slight differences in egg production which existed between
calcium levels were not statistically significant. However, a
difference in egg production was noted in Experiment V (Table
XV). With a 3.25 percent calcium level egg production was found
to be significantly greater than all other treatments. Taking
into consideration that the 2.75 and 3.75 percent calcium
levels did not similarly affect egg production, one is led to
question the significance of the response noted here.

These results therefore are in agreement with results re-
ported by Petersen et al. (65, p. 23) who indicate that high
calcium levels of 4.50 and 5.25 percent did not result in re-
duced egg production. It should be noted that no difference for
egg production was obtained from 1 percent calcium lactate in
contrast to the reports of Worden (94, p. 179-180), Kent et al.
(50, p. 9) and Gibson (34, p. 15) who indicate that the addition
of 1 or 2 percent calcium lactate to a layer's ration resulted
in a significant increase in egg production and/or the efficiency
of the ration in terms of numbers of eggs per kilogram of food
consumed.

In general egg weight, feed consumption, body weight and
mortality were not materially influenced by any dietary treat-
ment. There is very little variation between groups that can
be attributed to calcium levels in the ration.

SUMMARY

Several experiments were conducted to study the calcium requirement as related to certain reproductive characters of layers with particular reference to egg shell thickness. Both dwarfs and normal size Oregon Agricultural Experiment Station White Leghorn pullets were used. Variables employed to improve egg shell thickness were varying calcium levels from 2.25-3.75 percent with normal size hens and calcium-phosphorus levels of 2.25-0.6 to 3.0-0.9 percent with dwarf hens and their normal size sisters as well as oleandomycin and calcium lactate additions to the diet for normal size hens and ascorbic acid for dwarf hens. All experiments were conducted under natural environmental conditions. Egg shell thickness was measured by the specific gravity technique, measurements being taken during three successive days for normal size hens and four successive days for dwarf hens at the end of each 28-day period throughout the trials. Egg production, egg weight, feed consumption, body weight gains and mortality were noted.

Normal size layers in individual cages and on floor pens fed differing calcium levels produced thicker shells at levels above 2.25 percent calcium in the ration. A calcium level of 2.75 in the diet proved adequate for optimum egg shell thickness. No significant ($p < 0.01$) differences are apparent between 2.75 and 3.25 or 3.25 and 3.75 percent calcium levels. It has

been calculated by using actual data on feed required per dozen eggs that layers require calcium level ranging from 2.77 and 2.91 percent. For practical purposes 3.0 percent calcium may be recommended to provide some margin of safety. Addition of oleandomycin resulted in improving egg shell thickness with 2.25 percent and 3.0 percent calcium in the diet but to a greater extent with the higher calcium level. Three percent calcium level with oleandomycin resulted in highly significant effect on egg shell thickness. A significant increase in ash content of day-old chicks was obtained in the presence of oleandomycin and the higher calcium level. Two and one-quarter percent calcium made up in part with 1 percent calcium lactate in the laying ration did not increase or decrease egg shell thickness or ash content in metatarsus of day-old chicks. Blood calcium-phosphorus of layers fed differing calcium levels with or without oleandomycin did not show significant differences due to dietary treatments.

The data for the dwarfs indicate that the addition of ascorbic acid (10 mg./lb.) was without significant effect in improving egg shell thickness. Increasing the calcium-phosphorus level from 2.25-0.6 to 3.0-0.9 percent resulted in a marked improvement in egg shell thickness for the dwarf layers. A slight improvement was also obtained from the higher mineral levels with the normal size layers. On the basis of calculated calcium needs the data suggest that the dwarfs may have a somewhat higher physiological requirement for calcium than their normal

sisters. Doubling the vitamin-trace-mineral supplement in the 15 percent protein diet appears to improve egg shell thickness in the dwarfs to a greater extent than their normal sisters. This may indicate that a greater need exists for nutrient(s) contained in a vitamin-trace-mineral supplement. No beneficial effect was noted in the ration containing 18 percent protein.

Egg production, egg weight, feed consumption, body weight and mortality for the normal size layers were not consistently affected by any of the dietary treatments. For the dwarf hens egg production was improved 15 percent with high calcium-phosphorus levels. However, the dwarfs receiving the higher mineral level still laid 6 percent fewer eggs than the normals fed the lower mineral level. Feed consumption was increased 4 percent for the normals and 8 percent for the dwarfs fed high calcium-phosphorus levels. The dwarfs receiving the high calcium level consumed 30 percent less feed than the normals fed the low calcium level. Blood calcium and phosphorus of normal and dwarf hens fed high calcium-phosphorus levels appear to contain slightly higher blood calcium and phosphorus. However, in view of the variability encountered between replicates it is doubtful these observations are meaningful.

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