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Effect of reducing dietary anions (phosphate and chloride) on production characteristics of layers

Sarah Sykes Lambert

To the Graduate Council:

I am submitting herewith a thesis written by Sarah Sykes Lambert entitled "Effect of reducing dietary anions (phosphate and chloride) on production characteristics of layers." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

James K. Bletner, Major Professor

We have read this thesis and recommend its acceptance:

H.V. Shirley, M.C. Bell

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

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James K. Bletner, Major Professor

We have read this thesis
and recommend its acceptance:

H. V. Shirley

M. C. Bell

Accepted for the Council:

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EFFECT OF REDUCING DIETARY ANIONS (PHOSPHATE AND CHLORIDE) ON PRODUCTION CHARACTERISTICS OF LAYERS

A Thesis

Presented for the

Master of Science

Degree

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Sarah Sykes Lambert

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ABSTRACT

The purpose of this experiment was to determine the response of a single strain of high producing Single Comb White Leghorn-type hens to diets containing phosphorus at levels of 0.4 and 0.8 percent each with sodium being fed at a level of 0.25 percent in all the diets but with chlorine at levels of 0.36 or 0.16 percent.

The higher level of chlorine was attained through additions of salt (NaCl) to the basal diet. Furthermore, two low chloride sodium sources were added to the basal diet to reduce the dietary chlorides to the 0.16 percent level. Sodium bicarbonate served as one of these low chloride sources with a mixture of various sodium compounds serving as the second. This latter source was provided by Syntex Agribusiness, Inc. and was composed ofaa mixture of sodium bicarbonate, sodium sulfate, sodium propionate and 1.00 percent inert ingredients to contain 27.0 percent sodium.

Two trials were conducted, the first of 16 weeks duration and the second of 14 weeks duration. Results were subjected to analysis of variance. There appeared to be differences in response to dietary treatments as measured by shell quality, egg weight and body weight gain, but, these differences proved to be statistically nonsignificant. Neither were significant differences found between dietary phosphorus or low chloride sodium sources for egg production, feed consumption, percent mortality, or initial body weight.

Consistent trends were found between shell quality and dietary levels of both phosphorus and low chloride sodium sources. Feeding 0.4 percent phosphorus as compared to 0.8 percent resulted consistently in shells with higher specific gravity scores. Feeding the sodium source mixture to hens

resulted in higher quality shells than were obtained by feeding either salt or sodium bicarbonate. Also, the hens fed the sodium source mixture in combination with 0.4 percent phosphorus produced eggs with specific gravity scores higher than those produced with any of the other diets.

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CHAPTER I

INTRODUCTION

Strong egg shells are very important to the marketing of eggs. Each year the poultry industry loses millions of dollars because of egg shells cracked or broken during handling. Although much study has been given to improving shell thickness and/or shell quality, many questions remain unanswered and many problems remain unsolved.

For profitable egg production, the proper functioning of the shell forming organs must be maintained. Shell formation takes place primarily in the uterus of the hen. After ovulation, it takes about 4 1/2 hours for the egg to reach the uterus where it remains for 19 to 20 hours in order that shell deposition may be completed (Sturkie, 1976).

The egg shell is composed almost entirely of calcium carbonate. Therefore, shell calcification requires an adequate supply of calcium ions in the uterus or shell gland and the presence of carbonate ions in the shell gland fluids to form the calcium carbonate. The major source of the carbonate ions is blood carbon dioxide, however, blood bicarbonate is also important as a source of these ions (Card and Nesheim, 1976 and Sturkie, 1976). The calcium ions may be supplied through dietary sources and through the mobilization of bone calcium (Candlish, 1971 and Sturkie, 1976).

Numerous studies have shown that shell formation may be affected by those factors that influence the acid-base balance of the blood (Card and Nesheim, 1976). For example, factors that cause a reduction in blood bicarbonate ions (metabolic alkalosis) interfere with shell calcification as do factors that cause an excess of hydrogen ions in the blood (metabolic acidosis).

Nutrition of the hen is a major factor in maintaining the proper acid-base balance. Previous work by many researchers has indicated that reducing the total dietary level of phosphorus of the laying diet to 0.4 percent generally improves egg specific gravity. Just why lowering the phosphorus level of the diet improves shell quality is not known but it is probably associated with the acid-base balance. Studies by other researchers suggested that lowering the chloride intake of layers will also result in improved shell quality. This effect is the result of changes in the acid-base status making bicarbonate ions readily available for shell formation. Thus, the question was raised as to the effect of lowering the dietary levels of the two anions chloride and phosphate on certain production parameters of laying hens.

Therefore, experiments were designed to study the response of caged layers to diets containing 0.4 percent or 0.8 percent phosphorus each with chloride at 0.36 or 0.16 percent of the diet and a constant 0.25 percent sodium level.

CHAPTER II

LITERATURE REVIEW

I. FORMATION OF THE EGG SHELL

General

The egg shell is composed primarily of inorganic matter. Romanoff and Romanoff (1949) estimated the ash content of the shell to be approximately 95 percent of the total ash of the egg. Calcium salts form 98 percent of this inorganic content. The remainder is composed of small amounts of phosphorus and magnesium salts with trace amounts of iron and sulfur. Therefore, shell formation in the uterus of the hen could be described as the deposition of these mineral salts around the egg.

Romanoff and Romanoff (1949) also observed that three gradations were evident during formation of shell layers. There was a greater share of magnesium and phosphorus compounds in the inner portion; whereas, the outer portion was composed of nearly pure calcium carbonate.

After examination of the shell's composition and mode of formation, Simkiss (1968) concluded that two criteria had to be met for shell formation to take place. There had to be sites present for crystal nucleation and there had to be available a supersaturated solution of calcium and carbonate ions.

Changes in Anion:Cation Ratios During Shell Calcification

It has been established that the formation of the egg shell requires a supply of calcium ions to the shell gland and the presence of bicarbonate ions in the shell gland fluids. These must be present in sufficient amounts to form calcium carbonate of the egg shell. For a continuous supply of these ions to be present, large changes in the ion content of the body fluids occur. This phenomenon has been observed in both plasma and in fluids of the shell gland.

Blood plasma has been examined extensively for changes in its ion content during shell deposition. Feinberg, Hughes and Scott (1937) found that plasma calcium remained relatively constant during shell formation. Peterson and Parrish (1939) supported this observation by showing a 30 percent increase in plasma phosphatase and an increase in plasma inorganic phosphates of 15 to 39 percent at the time of shell deposition. Plasma chlorides were found to be consistently lower in layers as compared to non-layers (Common, 1941).

Changes in the ion content of the shell gland fluids were observed during shell formation by El Jack and Lake (1967) and Sauveur and Mongin (1971), as cited by Sturkie (1976). They found that sodium ion concentrations decreased from 143 meq/l at the onset of shell formation to 40 meq/l just before oviposition. Potassium concentrations increased from 8 meq/l to 68 meq/l and calcium remained relatively constant.

El Jack and Lake (1967) summarized their observations by reporting that sodium is the major cation present in plumping fluids 6 to 12 hours after the egg enters the uterus of the hen; calcium is the cation in greatest proportion in oviposition fluids; and the anions of chloride and carbonate are about equal in plumping fluids.

From a review of these observations, it seems that factors influencing the acid-base balance of the bird could also affect the processes of shell formation.

II. INTERACTION OF THE ACID-BASE BALANCE AND EGG SHELL QUALITY

Discovery of a Relationship

Many researchers have alluded to the hypothesis that a relationship exists between egg shell formation and acid-base balance in the laying hen.

Common (1941) reported that shell secretion apparently involved the metabolism of considerable amounts of fixed base. Due to a deficiency in plasma bicarbonates, alteration of the plasma alkali reserve took place making the acid-base equilibrium more alkalotic in the laying hen. This discovery was further studied by Gutowska and Mitchell (1945). Using bicarbonate ions from the blood of layers, they found that such ions were converted in the uterus to carbonate ions and were thus made available for shell calcification. This suggested a possible role of the acid-base balance in shell formation.

Then in the 1960's a series of experiments were conducted to examine and identify the mechanisms for regulation of shell calcification through the acid-base balance. Helbacka, Casterline and Shaffner (1963), Mueller (1966) and Anderson (1967) noted that changes in the status of acid-base equilibrium, measured as variation in blood pH, were definitely related to egg shell formation.

The first researchers to show a direct relationship were Mongin and Lacassagne (1966). They reported that as the egg entered the uterus, or shell gland, the pH of the veinous blood dropped from 7.53 to 7.41 while serum bicarbonate decreased in concentration from 31.5 meq/1 to 20.7 meq/1. The drop in bicarbonate also observed by Common (1941), was found to be accompanied by a decrease in pCO_2. These two effects maximized at 22 hours after the egg entered the uterus and resulted in a condition of metabolic acidosis.

Metabolic Acidosis

Metabolic acidosis is recognized as the prevailing condition in the uterus during the process of shell calcification. It is described by Mongin (1968) and Brobeck (1973) as a decrease in blood pH due to bicarbonate elimination. The result is a primary bicarbonate deficiency.

While studying the characteristic fall in uterine pH, Ogasawara, VanKrey and Lorenz (1964) proposed that the shell gland could regulate these changes to a limited extent.

Two mechanisms have since been recognized to be uitlized in the laying hen for pH regulation. The first is through increases in pulmonary ventilation, and the second is through increases in the excretion of ammonium salts and acids.

The basis of respiratory regulation is regulation of pCO_2 (Mongin, 1968 and Brobeck,1973). By increasing carbon dioxide exhalation, the carbonic acid concentration of the uterus is decreased. This in turn helps to elevate the bicarbonate : carbon dioxide ratio and bring the pH more toward the normal value.

The mechanism of renal regulation was explained by Anderson (1967). Noting that urine pH consistently decreased with the occurrence of metabolic acidosis, he found that there was an increase in urinary phosphates and complete reabsorption of bicarbonates. Mongin (1968) and Brobeck (1973) supported these observations with a description of the renal mechanism.

Reabsorption of filtered bicarbonates, regeneration of bicarbonates and excretion of ammonium salts and titratable acids restore the pH to normal levels and replenish the plasma bicarbonate levels.

Mueller and Leach (1974) found that with the occurrence of metabolic acidosis, egg shell thickness was significantly reduced. Therefore, the importance of maintaining these regulatory mechanisms and the proper acid-base equilibrium is quite evident.

Metabolic acidosis which is consistent with shell formation, must be controlled to avoid its interference with calcium carbonate deposition.

III. DIETARY REGULATION OF THE ACID-BASE BALANCE AND EGG SHELL DEPOSITION

Calcium and Phophorus

General. The relationship between calcium and phosphorus has been well established. It seems that the calcium:phosphorus ratio, however, is of relatively little importance in the laying hen. Singsen (1966) and Summers, Grandhi and Leeson (1976) reported that wide variations may exist in this ratio with little or no effect on the hen. They stated that the requirement for each of these minerals should therefore be treated independently.

Calcium requirement. The level of dietary calcium has a tremendous influence on the quality of the egg shell. At this time the National Research Council (1971) recommends that 2.75 percent of the diet be fed as calcium to meet the nutritional requirements of the laying hen.

In early studies, Miller and Bearse (1934) found no significant difference in shell quality when they fed diets containing 2.22 and 3.00

percent calcium. Gutowski and Parkhurst (1942) also observed no significant differences in shell quality with calcium levels of 2.35, 2.77, and 3.95 percent.

Evans, Carver and Brant (1944) found 3.0 percent calcium to be better than 2.25 percent for maintaining egg shell quality. Petersen et al. (1960) agreed with this observation stating that a level of 2.25 percent calcium was not sufficient to meet production and shell quality requirements.

Calcium at a level of 3.0 percent was found to be adequate for meeting production requirements by Arscott et al. (1962) and Cox and Balloun (1968). They further reported that this level of calcium resulted in thicker shells, higher production rates, and higher specific gravities than did a 2.25 percent level.

Hulan and Nikolaiczuk (1971) attempted to summarize this variation in recommendations. They indicated that 2.66 to 2.70 percent calcium is adequate to maintain production rates of 75 to 80 percent but that 2.70 to 3.15 percent calcium would ensure better egg shell quality.

Singh, Bletner and Goff (1971) and Shirley (1974) concurred by suggesting that 3.5 percent calcium is better for production in layers.

Phosphorus requirement. The phosphorus requirement of the laying hen has been studied extensively. Much like the research on calcium requirements, the results of these studies have been variable. The general consensus appears to be in support of the National Research Council (1971) recommendation that 0.6 percent total phosphorus is needed by the laying hen, a portion of which must be supplied in the form of inorganic phosphates.

The results of several experiments support a 0.4 to 0.6 percent level of total dietary phosphorus as sufficient for the hen in high production. O'Rourke, Phillips and Cravens (1955), Pepper et al. (1959), Singsen et al. (1962), Arscott et al. (1962), Hunt and Chancey (1970), Singh, Bletner and Goff (1971), Ademosun and Kalango (1973), Damron, Eldred and Harms (1974), Shirley (1974), and Summers, Grandhi and Leeson (1976) found this range of phosphorus to be adequate for maximum production.

The amount of phosphorus present in the egg shell could be supplied by a dietary level of only 0.09 percent phosphorus. Sunde (1963) concludes that more phosphorus is needed by the hen because of the importance of this mineral in numerous metabolic reactions.

Recommendations for dietary calcium and phosphorus. The real relationship between calcium and phosphorus in the diet seems to be explained in terms of low versus high levels of phosphorus in conjunction with adequate levels of calcium.

Numerous researchers using calcium at a level near 3 percent of the diet found that low dietary levels of phosphorus, 0.3 to 0.6 percent, tended to improve or maintain shell quality, (Pepper et al., 1959; Walter and Aitken, 1962; Arscott et al., 1962; Sunde, 1963; Salman and McGinnis, 1968; Hunt and Chancey, 1970; Singh, Bletner and Goff, 1971; Mraz, 1972; Damron, Eldred and Harms, 1974; Scott, Antillon and Krook, 1976; and Najib, 1976). High dietary levels of phosphorus, 0.7 to 0.9 percent, tended to decrease shell quality.

After reviewing these articles, it appears that 0.4 to 0.5 percent total or 0.3 percent available phosphorus is adequate to maintain high levels of production and favorable shell quality.

Sodium and Chloride

Most of the sodium and chloride in commercial-type laying diets is added in the form of salt (NaCl). In this combination, these two elements are used by the hen to maintain the integrity of the body fluids. The National Research Council (1971) recommended that sodium be 0.15 percent of the diet of the layer. They made no recommendation for chloride in the diet but stated that this amount of sodium would be equivalent to 0.37 percent NaCl. It is in this form that chloride is supplied in the diet.

The close interrelationship of these two minerals was established through several studies. In a study with chicks, Leach and Nesheim (1963) observed that sodium excesses increased chloride deficiency symptoms and resulted in higher mortality rates. It was then conclusively reported that sodium and chloride requirements are interdependent. Nesheim et al. (1964) and Melliere and Forbes (1966) found that sodium could ameliorate the deleterious effects of chloride excesses and that chloride could ameliorate the effects of excess sodium.

These two minerals were found to be instrumental in regulation of the acid-base equilibrium in the laying hen. Hall and Helbacka (1959), Hunt and Aitken (1962) and Nesheim et al. (1964) found that additions of chloride ions produced a metabolic acidosis. They also concluded that such disturbances in the acid-base balance caused by excess HCl could be corrected by feeding sodium bicarbonate. This proposal was studied by Frank and Burger (1965) with the findings that sodium supplied via bicarbonate was not retained unless chlorides were restricted.

Nott and Combs (1966) observed that a 1:1 ratio of sodium to chloride was best in feed formulae. Support was given to this work by Cohen, Hurwitz and Bar (1972) and Hurwitz et al. (1973). By holding dietary sodium levels constant and adding various chloride supplements, they were able to produce a dietary acidosis. By holding dietary chlorides constant and adding various sodium sources, dietary alkalosis was produced. Blood pH and bicarbonates were found to be sigmoidal functions of the dietary ratio of sodium to chloride. Therefore, they suggested that a ratio of unity was required for maximum production and feed consumption.

Bicarbonates

Plasma bicarbonates have been recognized to be of major importance in formation of the calcium carbonate deposited as the egg shell. Gutowska and Mitchell (1945) explained the mechanism of bicarbonate conversion. They found that bicarbonates from the blood are broken down into carbonate ions. The carbonate ions can then be complexed with calcium and used in the processes of shell formation.

The effects of bicarbonate feeding to improve egg shell quality were first examined and established by Frank and Burger (1965). They observed that sodium bicarbonate ingested from water resulted in a 6.5% increase in egg shell thickness. Howes (1967) and El Boushy, Simons and Wiertz (1968) also reported improvements in egg specific gravity with dietary additions of sodium bicarbonates.

Variable results were reported by Cox and Balloun (1968). In a six week experiment they found an increase in egg production but reported that thinner egg shells were produced after bicarbonate ingestion. Peirano and Bisson (1970) and Ernst et al. (1975) also got results that were inconsistent with previous workers. They reported that bicarbonate feeding produced no significant effects on egg shell quality.

From this variety of experimental observations, there is an indication that although bicarbonate is very important to shell formation, dietary sources of bicarbonates may not be available for use in shell formation.

IV. REGULATION OF SHELL QUALITY BY ADDITIONAL FACTORS

Environmental Temperature

Heat stress has been found to be of great importance in egg shell quality. Early workers established that environmental temperatures played a vital role in egg shell formation. This opened the door to a later explanation of the effect of heat stress on maintenance of the acid-base equilibrium in the laying hen.

Wilhelm (1940), Warren and Schnepel (1940), and Romanoff and Romanoff (1949) indicated that seasonal trends existed in shell thickness and that it was definitely correlated with environmental temperatures.

Helbacka et al. (1964) and Mueller (1966) found that high temperatures had a significant effect on the acid-base balance of the hen. Helbacka et al. (1964) observed that when birds were heat stressed and panted, there was an increase in blood pH and a reduction in shell thickness. Mueller (1966) found that at 34°C there was a 12 percent reduction in shell thickness in support of previous work. He then explained the physiological significance of heat stressing the layers as being the result of respiratory alkalosis. Such an explanation was consistent with the conclusions of researchers that were studying dietary bicarbonate effects.

Frank and Burger (1965), Howes (1967) and El Boushy, Simons and Wiertz (1968) noted bicarbonate feeding to be the most effective in heat stressed birds. They had explained such effects as the result of the establishment of dietary acidosis to overcome respiratory alkalosis.

Thus, bicarbonate feeding was compensating for the low plasma bicarbonate levels caused by hyperventilation. Simkiss (1968) noted that as a result of hyperventilation and heat stress, thinner shells were produced and blood bicarbonate levels were reduced. It was evident then that temperatures were also involved in acid-base balance.

Brobeck (1973) summarized the effects of high environmental temperature with a description of respiratory alkalosis. A primary carbon dioxide deficiency occurs; there is a decrease in carbonic acid as a result. Compensation is made by the renal tubules. They cease to excrete ammonium ions and acids. Reabsorption of bicarbonate ions is sharply diminished and considerable amounts of bicarbonate are excreted in the urine.

Respiratory alkalosis thus results in a marked decrease in shell quality.

Clutch Position

Several experiments have been conducted relating clutch position to thickness of the egg shell.

Berg (1945) observed considerable change in shell thickness from one day to the next in the same bird. He consistently found that the shells of the first and last eggs of three or more egg clutches were thicker than those of intervening eggs. This seemed to be partly due to position of the egg in the clutch but also suggested that the time interval involved in egg formation was important.

Atwood (1929), Haywang (1938) and Berg (1945) agreed that thickness of the shell within a clutch was highly correlated with the time interval involved in egg formation.

Experiments were then conducted to determine a pattern for shell thickness in a given sized clutch. Summarizing data on egg shell thickness, Wilhelm (1940), Romanoff and Romanoff (1949), and Tyler and Geake (1961) established such a pattern. In two egg cycles -- the second egg has a thicker shell. In three egg clutches -- the first and last eggs have thicker shells. In four or more egg clutches -- there is a gradual decrease in shell thickness until the last egg.

Roland, Sloan and Harms (1973) and Najib (1976) agreed that time of oviposition is related to shell thickness and found that the later in the afternoon the egg was laid, the better the eggshell with the possible exception of the first egg in the sequence. These observations were also in agreement with the pattern of shell thickness established by other researchers.

V. DETERMINATION OF SHELL QUALITY BY SPECIFIC GRAVITY

Various methods have been developed for measuring eggshell strength (Morgan, 1932; Almquist and Burmester, 1934; Stewart, 1936; Lund, Heiman and Wilhelm, 1938 and Voisey, 1975). The methods in use vary widely in cost, complexity and accuracy.

There are many characteristics of the egg used to determine shell strength. Some of these are shell thickness, weight loss, percent shell, shell deformation, weight per unit of shell and specific gravity. The most widely studied of these characteristics are the percent shell and shell thickness.

Morgan (1932) showed that a definite correlation existed between breaking strength and the percent shell of an egg. Shell thickness was then shown to be significantly correlated with the percent shell (Wilhelm, 1940). Indications were made that shell thickness could therefore be related to percent shell and thus egg quality.

Olsson (1934) as cited by Romanoff and Romanoff (1949) was the first to demonstrate that specific gravity was a good method of measure and a good indicator of the percent shell. Since that time, egg specific gravity has become one of the most widely used methods for assessing shell thickness. Wells (1968) reported that the advantages of specific gravity are threefold; the egg remains intact, it is a rapid method of determination, and it is relatively inexpensive. Therefore, it is adaptable to large numbers of eggs.

The specific gravity method was described by Novikoff and Gutteridge (1949) as dipping the eggs in brine solutions having a specific gravity range from 1.062 to 1.102 with intervals of .004, the end point being the lowest specific gravity in which the egg floats.

Potts and Washburn (1974) compared four methods of measuring shell quality, namely, non-destructive deformation, breaking strength, shell thickness and specific gravity. Correlations were made between these methods with the findings that specific gravity was highly correlated (+.56---+.88) with shell thickness and with breaking strength (+.72--+.80). Due to the high positive correlation between breaking strength and shell thickness, they concluded that specific gravity is the best of the four methods tested for evaluating egg shell quality.

CHAPTER III

EXPERIMENTAL PROCEDURES

I. EXPERIMENTAL ANIMALS

All birds used in this experiment were maintained at the University of Tennessee's poultry research facility, Cherokee Farm. They were vaccinated for Marek's disease at one day of age and were vaccinated for Newcastle, infectious bronchitis and fowl pox diseases during the growth period. They were fed a coccidiostatic drug in the starting and growing diets until they reached 14 weeks of age.

In Trial I, 216 pullets of the Single Comb White Leghorn-type were used. These birds of the Babcock B-300 strain were hatched on June 22, 1975, and were raised in solid floored pens until they were about 10 months of age. They were fed a commercial-type laying diet beginning at approximately 6 months of age. They were transferred to laying cages on May 10, 1976 and continued on the laying diet until June 8, 1976. Only one bird was assigned per cage. These hens were then assigned to experimental lots according to specific gravity averages during the pre-experiment period and fed one of the six experimental diets from June 9 until the completion of the trial on September 28, 1976.

In Trial II, 324 S.C. White Leghorn-type hens were used. These Babcock B-300 strain birds were hatched on June 21, 1976. They were maintained in solid floored pens until they were approximately 6 months of age. At that time they were housed in laying cages at a rate of one bird per cage and were randomly assigned to lots. These birds were fed the six experimental diets from January 3 until May 6, 1977 when the second trial was completed.

II. EXPERIMENTAL DIETS

The same six diets were fed throughout both trials. The diets were calculated to contain 0.4 or 0.8 percent phosphorus each with sodium at a level of 0.25 percent of the diet but with chlorine levels of 0.36 or 0.16 percent. The composition of the variable and nonvariable portions of the diets is indicated in Table I.

Two low chlorine sodium sources were used to obtain the latter levels of chlorine. Additions to the basal diet of salt (NaCl) and either the Syntex sodium source or the sodium bicarbonate source increased the dietary level of sodium while limiting the dietary level of chlorine. Table II shows the sodium and chloride content of some sodium sources.

The Syntex sodium source was furnished by Syntex Agribusiness, Inc. and was composed of sodium bicarbonate, sodium sulfate, sodium propionate and 1.00% inert ingredients (Madsen, 1976). The sodium bicarbonate was supplied simply as baking soda.

Trial II diets were subjected to chemical analyses and were found to be reasonably consistent with the values calculated. Calcium content was checked by the atomic absorption spectrophotometry method (Perkin-Elmer, 1968). Phosphorus content was checked by a spectrophotometric procedure according to A.O.A.C. (1975) and crude protein was determined by the Kjeldahl method of analysis (A.O.A.C., 1975).

III. EXPERIMENTAL DESIGN

This experiment was designed to determine the response of a single strain of S.C. White Leghorn-type layers to diets containing 0.4 and

TABLE 1

VARIABLES AND NONVARIABLES USED IN EXPERIMENTAL DIETS

Feedstuff	Diets: 0.4% Phosphorus, 0.36% Chlorine	0.4% Phosphorus, 0.16% Chlorine	0.8% Phosphorus, 0.36% Chlorine	0.8% Phosphorus, 0.16% Chlorine
Nonvariables	percent			
Soybean meal, 48.5%	17.300	17.300	17.300	17.300
Fishmeal (menhaden)	2.500	2.500	2.500	2.500
Alfalfa meal, 17%	5.000	5.000	5.000	5.000
Vitamin mix[a]	.500	.500	.500	.500
Manganese	.025	.025	.025	.025
Variables				
Yellow corn[b]	66.801	66.651	65.895	65.745
Defl. rock phosphate	.204	.204	2.370	2.370
Limestone, 38% Ca	7.170	7.170	5.910	5.910
Salt[c]	.500	.150	.500	.150
Sodium sources[d]	-----	.500	-----	.500
Total	100.000	100.000	100.000	100.000
Calculated Analysis				
Crude protein, %	16.93	16.92	16.85	16.84
Metabolizable energy, Kcal/kg	2832.00	2827.00	2801.00	2796.00
Calcium, %	3.00	3.00	3.00	3.00
Phosphorus, %	.40	.40	.80	.80
Sodium, %	.25	.25	.25	.25
Chlorine, %	.36	.16	.36	.16

[a]Vitamin mix supplied the following amounts per kg of diet: 2498 I.U. of vitamin A, 3000 I.C.U. of vitamin D_3, 4.59 mg of riboflavin, 25.00 mg of niacin, 441 mg choline, 4.76 mg pantothenic acid, and 0.66 ug of vitamin B_{12}.

[b]Corn and limestone were included as variables because their portions were varied to compensate for the difference in phosphorus content.

[c]Salt was included as a variable to compensate for differences in chlorine content.

[d]Supplied by the Syntex sodium source or by sodium bicarbonate.

TABLE II

SODIUM AND CHLORIDE CONTENT OF
SEVERAL SODIUM SOURCES

Compound	Sodium	Chloride
	percent	
Salt	39.3	60.6
Sodium bicarbonate	27.4	----
Sodium propionate	23.9	----
Sodium sulfate	32.4	----
Syntex sodium source[a]	27.0	----

[a]Composed of sodium bicarbonate, sodium sulfate, sodium propionate and 1.00% inert ingredients (Madsen, 1976).

0.8 percent phosphorus each with sodium at 0.25 percent but chlorine at 0.36 and 0.16 percent of the diet.

The birds and diets were assigned to lots on the basis of a complete randomized block design (Sokal and Rohlf, 1969). Each lot consisted of nine cages containing one hen each. In Trial I each of the six diets was fed to four replicate lots of hens and in Trial II each diet was fed to six replicate lots of hens.

Data were collected for Trial I during four periods, each twenty-eight days in length. Trial I began June 9 and ended September 28, 1976. Trial II data were collected for three twenty-eight day periods for a fourth period of twenty-two days duration. Trial II began January 21 and ended May 6, 1977.

IV. METHOD OF DATA COLLECTION

The methods of data collection were identical for both trials. Egg production was recorded Monday to Friday for each individual bird and adjusted to percent production. Feed consumption was determined for each period by lots. Body weights were taken on individual birds at the beginning of each trial and again just before the trial ended. Environmental temperatures were monitored by a daily record of the high, low and present temperatures.

Eggs laid by all birds were collected three times weekly, Tuesday through Thursday, for specific gravity and egg weight determinations. These determinations were made on the day following the day the egg was laid.

The specific gravity was obtained by placing the previous day's eggs in a basket according to group number. The baskets were then immersed in salt solutions graded from 0 to 8 with 0 being the lowest specific gravity. The specific gravity score recorded for each egg was the number of solution in which the egg floated. The relative concentration and specific gravity scores used in this determination are shown in Table III. The specific gravity of the solutions were identical to those used in the random sample laying tests (Shirley, 1976).

Mortality records were kept by removing dead birds from their respective cages and recording the death on the daily egg production sheet. The birds that died during the experiment were submitted to the diagnostic laboratory for necropsy.

V. ANALYSIS OF DATA

The data were statistically analyzed by the analysis of variance procedure for a randomized block design according to Sokal and Rohlf (1969). This analysis of variance in which each phosphorus level-sodium source sub cell was considered a separate treatment was conducted according to the following model:

$Yij = u + ti + rj + (t \times r)ij$

Yij = an individual observation of egg production, egg quality and physiological parameters on the ijk^{th} pen of birds.

u = the overall mean, assuming equal subclass number.

ti = the i^{th} phosphorus - sodium level subclass (treatment), i = 1, 2, ...6.

ri = the j^{th} replication, j = 1, 2, ...4 in Trial I, j = 1, 2, ...6 in Trial II.

$(t \times r)ij$ = the interaction of treatment and replication.

TABLE III

SPECIFIC GRAVITY OF SALT WATER SOLUTIONS USED IN SPECIFIC GRAVITY DETERMINATIONS

Specific Gravity	Score[a]
1.068	0
1.072	1
1.076	2
1.080	3
1.084	4
1.088	5
1.092	6
1.096	7
1.100	8
(sink in 1.100 solution)	9

[a]Scores used in random sample laying test (Shirley, 1976). Eggs floating in the various specific gravity solutions are given the corresponding score except eggs which sink in the 1.100 specific gravity solution are scored 9.

CHAPTER IV

RESULTS AND DISCUSSION

I. EGG PRODUCTION

The effects of dietary phosphorus levels and of low chloride sodium sources on egg production have been examined with contradictory results. In this experiment no significant differences were found due to dietary treatment in either of the two trials.

The mean values given in Table IV show that egg production of hens was maintained at an adequate level by all diets. The 0.4 and 0.8 percent levels of phosphorus resulted in comparable levels of production, as shown in Table V. These results support the works of O'Rourke, Phillips and Cravens (1955), Pepper *et al.* (1959), Singsen *et al.* (1962), Walter and Aitken (1962), Salman, Ali and McGinnis (1969), Hunt and Chancey (1970), Singh, Bletner and Goff (1971), Mraz (1972), Ademosun and Kalango (1973), Damron, Eldred and Harms (1974), Shirley (1974), Bletner and McGhee (1975), Scott, Antillon and Mullenhoff (1975), Scott, Antillon and Krook (1976) and Summers, Grandhi and Leeson (1976) that dietary phosphorus of 0.4 to 0.8 percent will support normal production levels for high producing layers.

The mean values in Table VI also show that there were no significant changes in production due to feeding the low chloride diets with additions of sodium. This was in agreement with Frank and Burger (1965) and Ernst *et al.* (1975) who found no significant differences in production due to feeding of low chloride diets with sodium bicarbonate additions.

TABLE IV

SUMMARY OF DATA FOR EGG PRODUCTION AND QUALITY, FEED CONSUMPTION, MORTALITY AND BODY WEIGHT

Diet		Production	Egg weight	Specific gravity	Feed/ hen	Feed/ dozen	Mortality	Body weight	
Na source	%P	%	gm	score[a]	kg	kg	%	Initial gm	Gain gm
Trial I diets									
NaCl	.4	75.77	61.55	3.41	12.70	1.80	0	1766	+ 33
	.8	75.80	62.27	3.16	12.66	1.78	0	1781	+141
Syntex[b]	.4	73.70	60.29	3.48	12.02	1.75	5.56	1674	+ 44
	.8	75.94	61.55	3.27[c]	12.78	1.81	0	1721	+119
Bicarbonate	.4	78.76	62.36	3.35	12.60	1.71	2.78	1832	+102
	.8	76.87	62.60	3.14	13.29	1.85	2.78	1774	+141
Trial II diets									
NaCl	.4	81.46	58.32	4.01	12.57	1.75	0	1660	+159
	.8	81.82	57.84	3.96	13.02	1.80	1.85	1663	+198
Syntex[b]	.4	81.80	57.99	4.17	12.72	1.76	0	1647	+181
	.8	78.85	57.55	4.10	12.74	1.83	0	1671	+208
Bicarbonate	.4	80.52	58.18	3.99	12.70	1.79	1.85	1644	+123
	.8	79.38	58.01	3.93	12.73	1.82	0	1640	+224

[a]Scores used in the random sample laying test (Shirley, 1976).

[b]Sodium source composed of sodium bicarbonate, sodium sulfate, sodium propionate and 1.00% inert ingredients (Madsen, 1976)

[c]Includes deletion of certain data and adjustment of the remainder.

TABLE V

EFFECTS OF PHOSPHORUS LEVELS ON EGG PRODUCTION AND QUALITY, FEED CONSUMPTION, MORTALITY AND BODY WEIGHT

% Phosphorus	Production %	Egg weight gm	Specific gravity score[a]	Feed/hen kg	Feed/dozen kg	Mortality %	Body weight Initial gm	Body weight Gain gm
Trial I								
0.4	76.08	61.40	3.41	12.44	1.75	2.78	1757	+ 60
0.8	76.20	62.14	3.19	12.91	1.81	0.93	1759	+134
Trial II								
0.4	81.26	58.16	4.06	12.66	1.77	0.62	1650	+154
0.8	80.02	57.80	4.00	12.83	1.82	0.62	1658	+210
Combined trials								
0.4	78.67	59.78	3.74	12.55	1.76	1.70	1704	+107
0.8	78.11	59.97	3.60	12.87	1.82	0.78	1709	+172

[a]Scores used in the random sample laying test (Shirley, 1976).

TABLE VI

EFFECTS OF SODIUM SOURCES ON EGG PRODUCTION AND QUALITY, FEED CONSUMPTION, MORTALITY AND BODY WEIGHT

Sodium source	Production %	Egg weight gm	Specific gravity score[a]	Feed/ hen kg	Feed/ dozen kg	Mortality %	Body weight: Initial gm	Body weight: Gain gm
Trial I								
NaCl	75.79	61.91	3.29	12.68	1.79	0	1774	+ 87
Syntex[b]	74.82	60.92	3.38	12.40	1.78	2.78	1698	+ 82
Bicarbonate	77.81	62.48	3.25	12.95	1.79	2.78	1803	+122
Trial II								
NaCl	81.64	58.08	3.99	12.80	1.78	0.93	1662	+179
Syntex[b]	80.33	57.77	4.14	12.73	1.80	0	1659	+195
Bicarbonate	79.95	58.10	3.96	12.71	1.80	0.93	1642	+174
Combined trials								
NaCl	78.72	60.00	3.64	12.74	1.79	0.46	1718	+133
Syntex[b]	77.58	59.35	3.76	12.56	1.79	1.39	1679	+139
Bicarbonate	78.88	60.29	3.61	12.83	1.80	1.86	1723	+148

[a]Scores used in the random sample laying test (Shirley, 1976).

[b]Sodium source composed of sodium bicarbonate, sodium sulfate, sodium propionate and 1.00% inert ingredients (Madsen, 1976).

The results of this experiment are in opposition to the conclusions of Hunt and Aitken (1962), Helbacka et al. (1964), Howes (1967), and Cox and Balloun (1968) that feeding sodium bicarbonate resulted in higher production during the decline of lay or during heat stress and that chloride feeding would result in lower production. No explanation is evident for such contradictory results.

II. EGG WEIGHT

The effect of diets on the average egg weight observed in these two trials agree with the work of Miller and Bearse (1934), Pepper et al. (1959), Singsen et al. (1962), Walter and Aitken (1962), Salman, Ali and McGinnis (1969) and Hunt and Chancey (1970) in that egg weight was not significantly related to sodium bicarbonate ingestion.

Table IV gives the mean egg weights. There were no significant differences between dietary treatments. The average egg weights in Table VI show, however, that a trend existed in this experiment. Average egg weight was consistently lower with the Syntex diet and was about equal for the salt and bicarbonate diets.

III. SHELL QUALITY

Table IV gives the mean specific gravity scores for each treatment combination. No significant effects due to diets were noted among treatments. However, Tables V and VI show certain trends that may be important and that are consistent with other research. Feeding of 0.4 percent phosphorus as opposed to 0.8 percent resulted consistently in higher average specific gravity socres. Feeding the Syntex sodium scores resulted in relatively higher quality shells than were obtained by feeding salt or bicarbonate as sodium sources. Table IV indicates that hens fed the Syntex source in combination with 0.4 percent phosphorus produced eggs with specific gravity scores that were higher than those produced by hens fed any of the other diets.

These trends agree with Pepper et al. (1959), Walter and Aitken (1962), Arscott et al. (1962), Sunde (1963), Salman, Ali and McGinnis (1969), Hunt and Chancey (1970), Singh, Bletner and Goff (1971), Mraz (1972), Damron, Eldred and Harms (1974), Scott, Antillon and Krook (1976) and Najib (1976) that 0.3 to 0.6 percent dietary phosphorus tends to improve or maintain shell quality while high dietary levels of 0.7 to 0.9 percent decreases shell quality and with Peirano and Bisson (1970) and Ernst et al. (1975) that bicarbonate feeding produces no significant effect on egg shell quality.

Such trends fail to agree with the results reported by Frank and Burger (1965), Howes (1967) and El Boushy, Simons and Wiertz (1968) that feeding sodium bicarbonate with low levels of chloride would result in improvements in egg specific gravity. Such discrepancies in experimental results cannot be readily reconciled although environmental temperatures may have had some effect. Both the ventilation and insulation systems of the housing facility were inadequate to maintain desired temperature levels, this being especially evident in Trial II. For Trial I the average high temperature was 29°C and the average low temperature was 21°C. The mean of the high temperatures in Trial II was 24°C and the mean for low temperatures was 17°C. When the data on specific gravity scores and environmental temperatures were compared, no relationships could be determined. This indicates that the birds in this study were not subjected to heat stressing.

It may also be noted that specific gravity scores were higher for Trial II than for Trial I. This was due to age of the birds on experiment. The hens in Trial I were several months older than those in Trial II and, as expected, produced eggs with shells of a lower quality.

IV. FEED CONSUMPTION

Feed consumption was analyzed by two parameters, that of feed per hen and feed per dozen eggs. Table IV, page 24, shows that there were no significant differences among treatments.

Feed per dozen eggs is affected by both the rate of production and the body size of the hen. High producing hens consume more feed than low producers but consume less feed per dozen eggs. Since no significant differences were found in the amount of feed consumed per hen or in the rates of production and body weight, it is expected that the feed consumed per dozen eggs would not differ significantly.

These results are in agreement with the results of Pepper _et al_. (1959), Singsen _et al_. (1962) and Walter and Aitken (1962) that feed consumption is not affected by dietary phosphorus levels.

No data were found in the literature regarding the effect of dietary chloride levels on feed consumption.

V. MORTALITY

Mortality was shown to be significantly affected by replication in Trial I. This was probably due to the small number of birds sampled. Diet resulted in no significant effect on mortality as is shown in Table IV, page 24. Mortality could not be attributed to any specific cause and was not related to dietary treatment.

VI. BODY WEIGHT

Average initial body weight and average final body weight were used to analyze changes in body weight. Table IV, page 24, shows the average initial weight and the average gains in grams. No significant differences were found in initial weights indicating uniformity of the birds. Neither were significant differences found in body weight gains due to dietary treatment. This agrees with the findings of Hunt and Chancey (1970), Pepper et al. (1959) and Singsen (1962) that similar body weights were obtained by feeding 0.4 percent phosphorus and by feeding higher levels of dietary phosphorus.

Table V, page 25, indicates that relatively larger gains were consistently observed with the 0.8 percent phosphorus diets when compared to the 0.4 percent phosphorus diets. Walter and Aitken (1962) state that levels greater than 0.4 percent of dietary phosphorus are better for maintaining body weight in high producing layers. These trends support the work of Ademosun and Kalango (1973) and Najib (1976) that showed 0.6 percent phosphorus resulted in significantly higher body weight.

Table VI shows that the salt diet produced slightly lower gains than did the other two sodium sources. This would be due to the greater amount of chloride ions present in the salt diet. Low chloride diets promote higher gains in layers while excesses of dietary chloride will depress growth (Nesheim et al., 1964 and Hurwitz et al., 1973).

VII. CLUTCH POSITION

Statistical analyses were made on the specific gravity of the first and last eggs of the clutch in Trial II. Table VII shows these average specific gravity scores.

TABLE VII

AVERAGE SPECIFIC GRAVITY SCORES[a] FOR THE FIRST AND LAST EGGS OF THE CLUTCH

Diets: Na source	%P	First eggs	Last eggs
		average scores	
Dietary effects			
NaCl	0.4	4.26	4.34
Syntex[b]	0.4	4.29	4.28
Bicarbonate	0.4	4.18	4.23
NaCl	0.8	4.24	4.29
Syntex[b]	0.8	4.05	4.26
Bicarbonate	0.8	4.27	4.34
Sodium source effects			
NaCl	---	4.25	4.32
Syntex[b]	---	4.17	4.27
Bicarbonate	---	4.28	4.29
Phosphorus effects			
-----------	0.4	4.24	4.25
-----------	0.8	4.17	4.30

[a]Scores used in the random sample laying test (Shirley, 1976).

[b]Sodium score composed of sodium bicarbonate, sodium sulfate, sodium propionate and 1.00% inert ingredients (Madsen, 1976).

Since no significant differences were indicated among the treatment, it may be true that all improvements in the specific gravity scores had to be made in connection with the eggs from the middle of the clutch. Improvement in the middle eggs is highly desirable. The first and last eggs of the clutch have higher specific gravity scores than do the other eggs in a given clutch (Wilhelm, 1940 and Tyler and Geake, 1961). Thus, if middle eggs can be improved, they would have shells of a quality more comparable to that of the first and last eggs of the clutch. This could result in the reduction of losses incurred by handling and shipment of eggs.

VIII. CONCLUSIONS

Trends observed in this study support the theory that low levels of dietary phosphorus and substitution of low chloride sodium sources for part of the dietary salt may improve shell quality. Feed phosphorus at the level of 0.4 percent with the low chloride sodium sources resulted in higher quality egg shells than did the diets containing 0.8 percent phosphorus.

Since no significant differences were found for dietary treatments, it is indicated that cost may become the deciding factor for practical usage by the producer of sodium sources. If sodium sources constitute additional cost, the monetary benefit of improved shell quality might not outweigh the price for attaining it.

CHAPTER V

SUMMARY

This research was conducted to determine the response of a single strain of Single Comb White Leghorn-type hens to diets containing phosphorus at levels of 0.4 and 0.8 percent, each supplemented with salt (NaCl) to yield a chlorine level of 0.36 percent or with one of two low chloride sodium sources to replace some of the dietary sodium chloride and yield a chlorine level of 0.16 percent of the diets. Sodium bicarbonate was added as one of the low chloride sodium sources and a mixture of sodium salts was added as the second. The latter source was supplied by Syntex Agribusiness, Inc. and was composed of sodium bicarbonate, sodium sulfate, sodium propionate and 1.00 percent inert ingredients to yield a sodium level of 27.0 percent.

Response was determined through the parameters of egg production, egg weight, egg shell quality, feed efficiency, mortality and body weight.

Two studies were conducted using the six dietary treatments. Trial I ran for 16 weeks with the diets being fed to four replicate lots of nine birds each. Trial II ran for 14 weeks with the diets being fed to six replicate lots of nine birds each.

Measurements of specific gravity and egg weight were taken three times weekly during each trial. The results from the study were as follows:

1. Egg production was not affected by phosphorus level or sodium source.
2. Egg shell quality as assayed by specific gravity was not significantly affected by dietary treatment. Specific gravity scores were observed to increase when low phosphorus levels were fed and

were found to be higher than all other diets when 0.4 percent phosphorus was fed with the low chloride sodium mixture (Syntex).

3. Average egg weight was not significantly affected by diet but was found to be lowest when the sodium mixture was fed as the sodium source.
4. Feed consumption was not related to dietary treatment.
5. Mortality was not affected by diet.
6. Body weight as assayed by initial body weight and body weight gains was not significantly affected by dietary treatment. Gain was observed to be consistently higher for 0.8 percent phosphorus diets as compared to 0.4 percent phosphorus level diets.
7. The first and last eggs of the clutch were not affected by dietary treatment with respect to specific gravity.

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