THE EFFECTS OF ANTIBIOTICS IN THE LAYING RATION

UPON

EGG-SHELL QUALITY

A Thesis

by

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1. INTRODUCTION

The adaptation of the poultry industry to accommodate the needs of large scale production has resulted in the division of the industry into specialized units of meat and egg production. Egg production constitutes the major phase, from the aspect of both the percent of the total investment in the industry which is devoted to it and the number of flocks that is kept for egg production. It is to be expected then, that research study in the poultry field has until recently been devoted mainly to the problems associated with large-scale egg production. Problems of immediate practical importance, such as those of nutritional and genetic origin, devoted to increased annual production, have been investigated and in most cases solved.

Several problems of economic significance, however, are still outstanding, and among these the maintenance of a high standard of egg-shell quality is of prime importance. Shell quality characteristics include the texture and thickness of shell. The first two are important factors connected with consumer appeal. Shell thickness is of prime importance in that,

(i) the enormous breakage of eggs that occurs every year on farms and in marketing channels is due to a considerable extent to thinness of shell, and

(ii) chicks hatched from eggs with a low percentage of shell have a higher death rate during the brooding stage than those from eggs with a higher percentage of shell. Munro (31) and (32).

A. <u>A Review of Literature Pertaining to</u>

1) Factors directly affecting egg-shell thickness and related characteristics,

2) Antibiotics and calcium metabolism.

1) <u>Factors directly affecting egg-shell thickness and related characteristics</u>.

The factors which have been shown to be directly associated with thickness of shell may be discussed under the headings:

(a) Genetic

(b) Environmental I Nutritional

II Temperature

(c) Miscellaneous.

(a) <u>Genetic</u>

The inheritance of egg-shell thickness and factors associated with shell thickness have been demonstrated by numerous workers. Willard and Shaw (51), Romanoff (37), Baskett, Dryden and Hale (4), have indicated the heritability of such characteristics as shell percentages of total weight, shell porosity, thickness and breaking strength.

Taylor and Lerner (46) by selection were able to develop two lines of White Leghorn that differed significantly in the amount, thickness, and percentage of egg shell. The percentage of the total variation in egg-shell thickness that can be attributed to genetic factors was estimated by McClary and Lerner (28) to be from 15-30%.

(b) Environmental

I Nutritional

The shell of the hen's egg contains 5-9 grams of ash, of which the salts of calcium form more than 98%, according to Romanoff and Romanoff (36). These authors also observe that the total output of calcium by a hen producing 200 eggs a year is in the region of 0.5 kg. The necessity for adequate sources of available calcium for normal shell formation is therefore obvious.

Temporary drafts on the body reserves may be made to meet with the demands of egg production as illustrated by Romanoff and Romanoff (36), but the calcium for the shells of a series of eggs must ultimately come from the diet. Prolonged restriction of calcium in the diet results in the production of thin-shelled eggs according to Deobald, Lease, Hart and Halpin (15), and it may also be noted here that Evans et al. (16) observed that prolonged calcium deficiency conditions resulted in acute softening of the bone. Associated with calcium in the maintenance of normal egg-shell thickness are vitamin D and the minerals phosphorous and manganese.

Hendricks, Lee and Godfrey (19), Martin, Erickson and Insko (27) and several other workers have reported an increase of thickness of shell on supplying sources of vitamin D in the ration.

Carver et al. (11) reported that insufficient amounts of vitamin D resulted in inferior quality of egg shells.

Although Miller and Bearse (30) concluded that the quality of shell was not influenced by the level of phosphorous in the diet, work carried on at this laboratory suggests that a slight lowering of specific gravity of eggs occurs when low phosphorous rations are fed.

Lyons (25) has demonstrated the inferiority of shell quality when pullets were allowed insufficient amounts of manganese in the ration. An increase in the manganese allowance of the pullets elicited an immediate response illustrated by a marked improvement in shell quality.

II Temperature

Hays (20) observed that pullets attained their maximum egg-weight in February, a decline in weight occurring during the summer months.

Bennion and Warren (8), on the strength of the observation made by Hays, suggested that the seasonal fluctuations in egg-size in various parts of the U.S.A. may have been due to some effect of climate or temperature.

An experiment designed to prove this hypothesis yielded results which indicated a definite effect of temperature on egg-weight of birds held under controlled temperature conditions. In this experiment egg-weight was reduced 15-20% by application of high temperatures. All components of the egg were shown to decrease under such conditions, the shell and albumen showing considerably greater decrease than the yolk, in proportion to their weight. Bennion thought that this variation in the decrease of the component parts of the egg, indicated that the oviduct was probably more sensitive to high temperatures than the ovary.

Miller and Bearse (30) determined the shell percentages of eggs from December to October and found a consistent decrease, beginning March and extending throughout the summer period.

Warren and Schnepel (48), assuming that the variation of the percentage of the egg which is shell was closely associated with shell thickness, studied the effect of varying air temperatures on egg-shell thickness. Egg-shell measurements were made with a screw-type caliper capable of being read to .0005 inch. The results obtained clearly demonstrated a striking reduction in egg-shell thickness when hens and pullets were subjected to high temperature conditions. The data obtained also suggested that high humidity tended to accentuate the depressing effects of high temperature on shell thickness.

Hughes et al.(21) observed that the blood plasma of the laying hen contained about twice as much calcium as that of the non-laying bird.

Warren and Schnepel (48), while engaged in the work on egg-shell thickness previously mentioned, made some blood serum calcium determinations and reported that their limited observations seemed to point to a reduction in blood calcium content in about the same proportion as the reduction in shell thickness, when the birds were subjected to high temperatures.

Conrad (14) showed a definite effect of high temperatures on blood calcium in the laying hen. He reported a decrease of blood calcium level of from 20% to 30% when the environmental temperature was increased from 70°F to 90°F.

Based on the observations of the workers mentioned, it would appear that perhaps the primary effect of high temperatures is connected with a disturbance of normal calcium metabolism, resulting in a lowering of the blood calcium level. The decrease in egg-shell thickness would under these circumstances be a secondary effect, attributable to the lowered level of blood calcium.

Increased allowances of calcium or vitamin D will not result in a comparable increase in blood calcium and shell thickness under adverse temperature conditions. Tyler (48) has shown that on already adequate calcium diets additional amounts of calcium did not increase the shell thickness beyond normal. He suggested that a diet high in calcium may not be capable of increasing the blood calcium level above a particular threshold and hence will have no further effect on shell thickness.

(c) Miscellaneous

Factors such as the effect of position of egg in the clutch, as related to the thickness of the egg have been investigated by Wilhelm (50) and Berg (7). Wilhelm reported little decrease between the first and last eggs of the same clutch, but Berg observed that the shell of the second egg of two-egg clutches was thicker than that of the first egg of the clutch.

Disease conditions in the flock will affect shell thickness. Berg, Bearse and Hamilton (6) observed that Newcastle disease caused some birds to lay thin-shelled eggs.

Scott, Jungherr and Matterson (40) demonstrated the effect of sulfanilamide in reducing egg-shell thickness.

(2) Antibiotics and Calcium Metabolism

Common, Keefe, Burgess and Maw (13) observed that the increase of both serum calcium and serum riboflavin which follows the injection of estrogens into immature pullets was enhanced when aureomycin was included in the diet.

Migicovsky, Neilson, Gluck and Burgess (29) reported that the inclusion of penicillin in the diet of chicks, led to a marked increase in the absorption of radio-active calcium.

Lindblad, Slinger, Anderson and Motzok (23) showed that aureomycin tended to improve the utilization of calcium and phosphorous, so that in the presence of the antibiotic, the requirements of these minerals for optimum growth was reduced. Ross and Yacowitz (38) suggested, from the results of their observations, that penicillin decreased the vitamin D requirements for normal bone calcification. They fed a corn-soya bean basal ration deficient in vitamin D, but complete in all other vitamins and minerals known to be required components in a normal chick ration. Vitamin D in the form of activated sterol was added to the basal ration at levels of 4, 8, 30 and 600 I.U. per 100 gm., with and without the addition of procaine penicillin. At the end of three weeks no apparent effect of the antibiotic on the vitamin D requirement for growth was observed. At the 8 and 30 I.U. levels of vitamin D, a statistically significant increase in the percent bone-ash was observed in the presence of penicillin. No explanation was offered for failure of penicillin to affect calcification at the higher and lower levels of supplementation.

Gabuten and Shaffner (18) studied the effect of a series of compounds, among them penicillin, on the specific gravity of eggs. He noted that penicillin maintained or slightly increased the specific gravity of eggs, which normally declines during the summer months, and as the production year advances. He also reported that the calcium level in the blood serum and the shell-breaking strength of the eggs were higher in the penicillin-fed birds than in a control lot.

Sturkie and Polin (45) observed no effect of penicillin on the mobilization and utilization of calcium when chickens were injected with single large doses of antibiotic. They however noted the fact that the positive results obtained by other workers have been under conditions when the antibiotic was fed for an extended period of time and in relatively small amounts of from 10 to 30 ppm.

B. Object

The foregoing literature review gives an insight into the complexity of the egg-shell quality problem and the difficulties involved in adopting management practices to control all the factors associated with thin-shelled eggs, where the condition does exist.

The genetic and nutritional aspects are the more easily controlled. The effect of temperature on egg-shell thickness has posed a problem, in that the actual mechanism involved in the lowering of egg-shell strength during the hot months, has not been discovered.

The observation that small amounts of antibiotics fed over a period of time are capable of influencing calcium metabolism, has suggested a practical answer to offset the deterioration of egg-shell quality during the summer months.

The object of this investigation has therefore been to test the effect of the antibiotics, penicillin, bacitracin and terramycin, on egg-shell quality in the laying hen, under normal management conditions. A study of the possible mechanisms involved in the effect of antibiotics on calcium metabolism has also been undertaken, with a view to explaining the relationship already observed by several workers.

II EXPERIMENTAL

A. General Experimental Procedures.

The experimental procedure adopted in this investigation may be divided into five sections as outlined below.

1. A preliminary investigation in laying batteries, the primary purpose of which was to investigate trends of the effect of the antibiotics used on egg-shell quality, as reported by several workers.

2. An investigation of the effect of the antibiotics on egg-shell quality under practical floor conditions of summer management.

On the basis of the results obtained from experiments (1) and (2) two further experiments (3) and (4) were undertaken as follows:

3. Investigation of the effect of the antibiotics on bone calcification in chicks when included in a diet deficient in vitamin D_3.

4. Investigation of the effect of the antibiotics on bone calcification of chicks when included in

   (i) low calcium diets

   (ii) low phosphorous diets.

To further investigate the results obtained in Experiment 4, the following experiment was conducted:

5. Investigation of the effect of antibiotics on egg-shell quality on hens fed a low calcium ration.

The experimental plan for each of the five experiments will be outlined separately and individual results and discussions for each experiment given under the appropriate headings.

B. Methods of analysis for the four experiments are outlined as follows:

1. Thickness of Shell

Measurements of egg-shell thickness were made with an ordinary screw-type caliper capable of being read to .0005 inch. The recorded thickness of shell ~~measurement~~ of one egg consisted of the average of 3 measurements of shell thickness, one at each end, and one on the side of the egg.

2. Specific Gravity

The method of specific gravity measurement~~s~~, as first suggested by Olsson (33), was adopted for making the measurements in this investigation. A series of salt solutions, ranging in specific gravity from 1.0590 to 1.0940 with graded intervals of .0035, was prepared by using a hydrometer graduated so that .001 could be read accurately and .0005 unit estimated. The salt solutions were kept in stoppered bottles, and were tested each week.

The eggs were dipped into the salt solutions and the specific gravity of each egg was recorded as that of lowest concentration of the salt solution in which it just floated beneath the surface.

3. Blood Serum Calcium Determination

The blood serum calcium determinations were made by titration of the serum with ethylenediamine tetra-acetate as suggested in the Sobel and Hanok Method (42). Two modifications to the procedure were used here:

(i) 4N NaOH buffer, as suggested by Banks (3), was substituted for the ethonolamine buffer as it was found that a better end point could be obtained by this modification.

(ii) A screened indicator as used by Knight (22) for the determination of the calcium content of water, was found to give a clearer colour change at the end point than the pink to purple change of the murexide indicator used by Hanok and Sobel.

Reagents

1. Standard Solution of Calcium (Stock)

2.0018 gm. of calcium carbonate ~~was~~ were dissolved in a minimal amount of 6N HCl. This was evaporated on a steam bath. The residue was washed into a 100 c.c. volumetric flask and made to volume with glass-distilled water. A working standard was prepared from this stock solution by diluting 1 ml. of the stock standard to 100 mls. This was the calcium standard used by Sobel and Hanok. It contained 8.015 mg. of calcium per 100 mls.

2. Ethylenediame Tetraacetate[1] (Disodium salt) Reagent

The working solution was made by dissolving .45 gms. of E.D.T.A. in a litre of distilled water. Approximately 1.7 mls. of this solution was required to titrate the calcium in 1 ml. of the working standard.

[1]Hereafter designated as E.D.T.A.

3. Knight's Screened Indicator

.200 grams of murexide, .500 grams of Napthol Green B and 100 grams of pure NaCl were ground together and thoroughly mixed.

4. 4N NaOH

Procedure for Standardization of E.D.T.A.

To 1 ml. of the standard calcium solution in a test tube, 2 ml. of 4 NaOH ~~was~~ were added. Two milligrams of the solid indicator were added, and titration of the standard carried on with the E.D.T.A. The end point of the titration was indicated by a change from a reddish colour to a definite blue.

Titration of the Serum

1 ml. of serum was used for titrating with the E.D.T.A. The procedure followed was as outlined above for the standardization of the E.D.T.A.

For keeping the serum agitated while titrating, air bubbles were introduced into the bottom of the test tube, by the use of a special pressure apparatus equipped with glass stirring points.

4. Bone Ash Determination

Bone ash determinations were made as per procedure outlined in A.O.A.C. (1)

The bones were cleaned of all adhering tissue and the fat removed by refluxing for 20 hours with hot alcohol and then for a further 20 hours with ethyl ether. The bones were then dried to a constant weight in a drying oven.

The dried bones were weighed so as to determine water-free, fat-free weight, and then were ashed for 4 hours at a temperature of 550 C.

C. Experiment 1

Trends in the effect of antibiotics on egg-shell quality in the laying hen

It was thought that the use of small numbers of birds held under battery conditions would serve as a basis for future work under conventional floor conditions of management. Consequently the following experimental plan was utilized:

(1) Experimental Plan

Sixteen New Hampshire X Barred Plymouth Rock hens were placed at random, in individual compartments of a laying battery.

A premixed basal ration consisting of laying mash 50% and scratch grain 50% was supplemented with antibiotics as follows:

Treatment 1: Basal ration and procaine penicillin (Merck)

Treatment 2: Basal ration and bacitracin (Commerical Solvents)

Treatment 3: Basal ration and terramycin hydrochloride (Pfezer)

A portion of the basal ration not supplemented with antibiotic was designated as Treatment 4.

The four treatments were assigned to the sixteen hens so that the experiment consisted of four replications of the four treatments.

A level of 10 p.p.m. of antibiotic supplementation was fed from March 7 to May 26 inclusive when the level was raised to 20 p.p.m. Feed, water, shell and grit were allowed ad libitum throughout the experimental period.

Eggs were collected daily for the purpose of obtaining egg-shell thickness and specific gravity measurements.

Specific gravity measurements were made at the end of each day. Blood serum samples were taken for analysis of blood calcium at the beginning of the experiment and at the end of each experimental period involving one level of the antibiotic.

(2) General Results and Discussion

The average shell thickness and specific gravity data under the two levels of antibiotic supplementation are outlined in Table 1.

In general it was observed that the average specific gravity and average shell thickness measurements were maintained at a higher level in the antibiotic-fed birds (Treatments 1, 2 and 3) than in the non-supplemented group, (Treatment 4).

Temperature conditions were not controlled throughout the experiment but were allowed to vary with the environmental conditions. A direct comparison between the two levels cannot therefore be made due to the seasonal temperature differences which existed during the two periods. Further, the increase in the antibiotic level was made to counteract what seemed to have been a refractory response to the antibiotics towards the end of the 10 p.p.m. level of supplementation. However, from an observation of the data, there is an indication that the effect of the increased supplement of antibiotic is small.

The average decline in shell thickness in the basal group between the first and second periods of treatment was from .0125 inches to .0122 inches, a difference of .003 inch. A somewhat similar decline occurred in the case of the bacitracin and terramycin groups. The penicillin supplemented group during the same period showed no change.

If the relatively low value of shell thickness at the

Table 1. Av. specific gravity and shell thickness of eggs from hens held under battery conditions at two levels of antibiotic supplementation.

Treatment	Bird no.	March 7 - May 26 (10 p.p.m.) No. of eggs	Av. S. G.	Av. shell thick. in.	May 27 - July 17 (20 p.p.m.) No. of eggs	Av. S. G.	Av. shell thick. in.
1. Penicillin	4	47	1.0835	.0135	28	1.0805	.0133
"	8	42	1.0800	.0119	24	1.0748	.0116
"	12	44	1.0868	.0139	29	1.0831	.0142
"	16	35	1.0818	.0125	31	1.0774	.0128
Av. of treatment			1.0830	.0130		1.0790	.0130
2. Bacitracin	5	33	1.0848	.0135	29	1.0796	.0133
"	9	21	1.0822	.0131	15	1.0779	.0130
"	12	52	1.0851	.0135	37	1.0797	.0135
"	15	30	1.0827	.0130	26	1.0778	.0127
Av. of treatment			1.0837	.0133		1.0788	.0131

Table 1. (contd.) Av. specific gravity and shell thickness of eggs from hens held under battery conditions at two levels of antibiotic supplementation.

Treatment	Bird no.	March 7 - May 26 (10 p.p.m.)			May 27 - July 17 (20 p.p.m.)		
		No. of eggs	Av. S. G.	Av. shell thick. in.	No. of eggs	Av. S. G.	Av. shell thick. in.
3. Terramycin	3	42	1.0827	.0128	12	1.0782	.0127
"	6	36	1.0826	.0134	35	1.0791	.0131
"	10	33	1.0860	.0139	23	1.0802	.0135
"	11	39	1.0848	.0137	23	1.0800	.0134
Av. of treatment			1.0840	.0135		1.0794	.0132
Basal	1	51	1.0793	.0107	14	1.0782	.0117
"	2	49	1.0819	.0129	29	1.0757	.0124
"	7	40	1.0884	.0142	11	1.0777	.0129
"	14	56	1.0801	.0120	24	1.0737	.0116
Av. of treatment			1.0824	.0125		1.0763	.0122

end of the first period precluded a further marked lowering of shell thickness during the second period, then what would appear to be a similar small decline in the case of Treatments 2 and 3 as noted above, in reality may have been due to the effect of an increased level of the antibiotic under conditions of increased temperature stress.

The specific gravity data for the different antibiotics exhibited the same trends, showing higher values than the control group, even in the second period when a general decline in specific gravity was noted.

The blood serum calcium levels under the different treatments appear in Table 2. The trends were similar, with the downward trend in blood calcium over the second period being marked only in the case of the unsupplemented group. The hens on the terramycin supplement actually showed a rise in blood calcium during that period.

The graphs in Figures 1 and 2 illustrate in a comparative way the specific gravity and shell thickness trends as discussed above.

THE EFFECT OF ANTIBIOTICS ON THE AVERAGE SPECIFIC GRAVITY OF EGGS BY WEEKLY PERIODS WHEN HENS ARE HELD UNDER BATTERY CONDITIONS

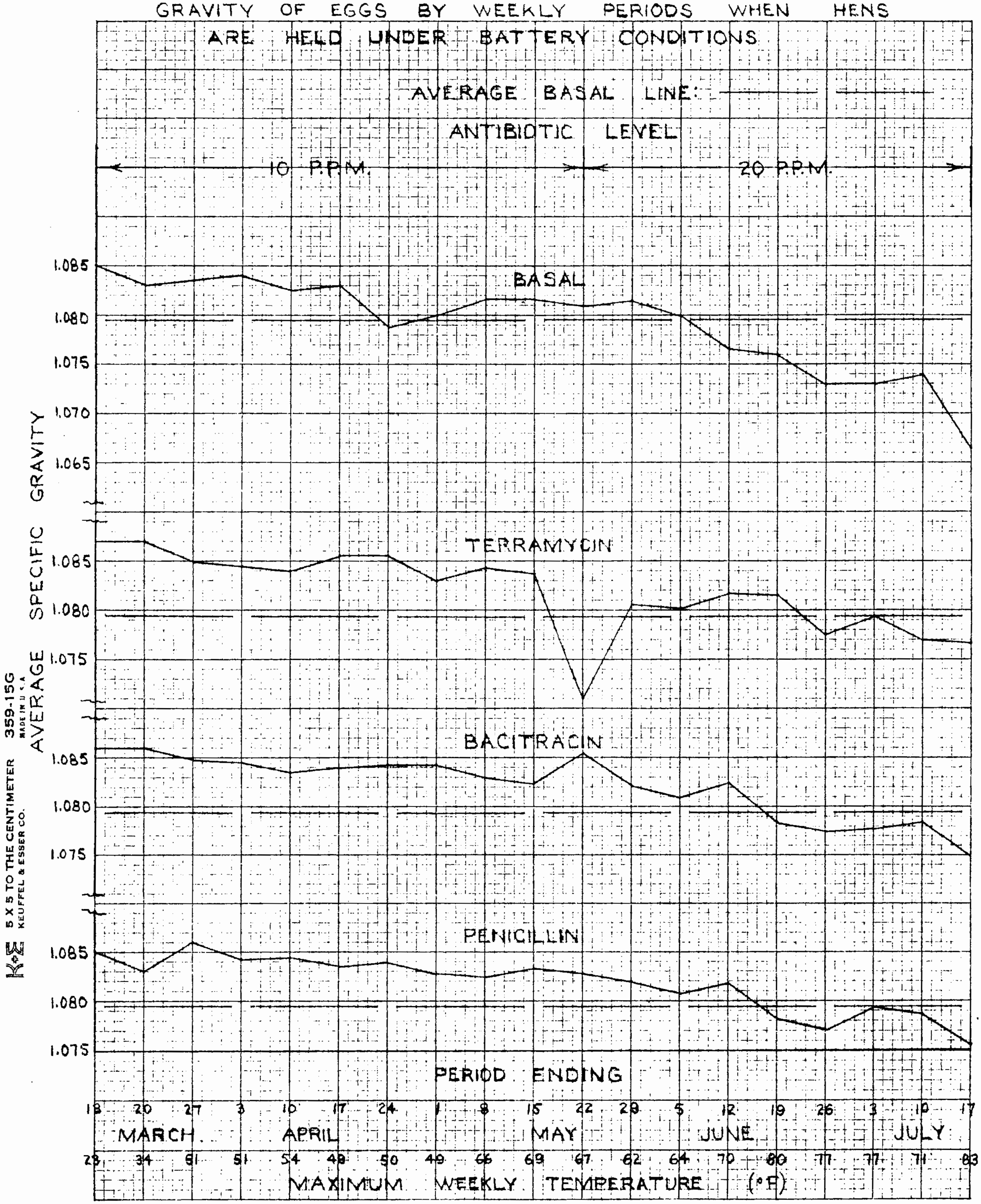

FIG. 1

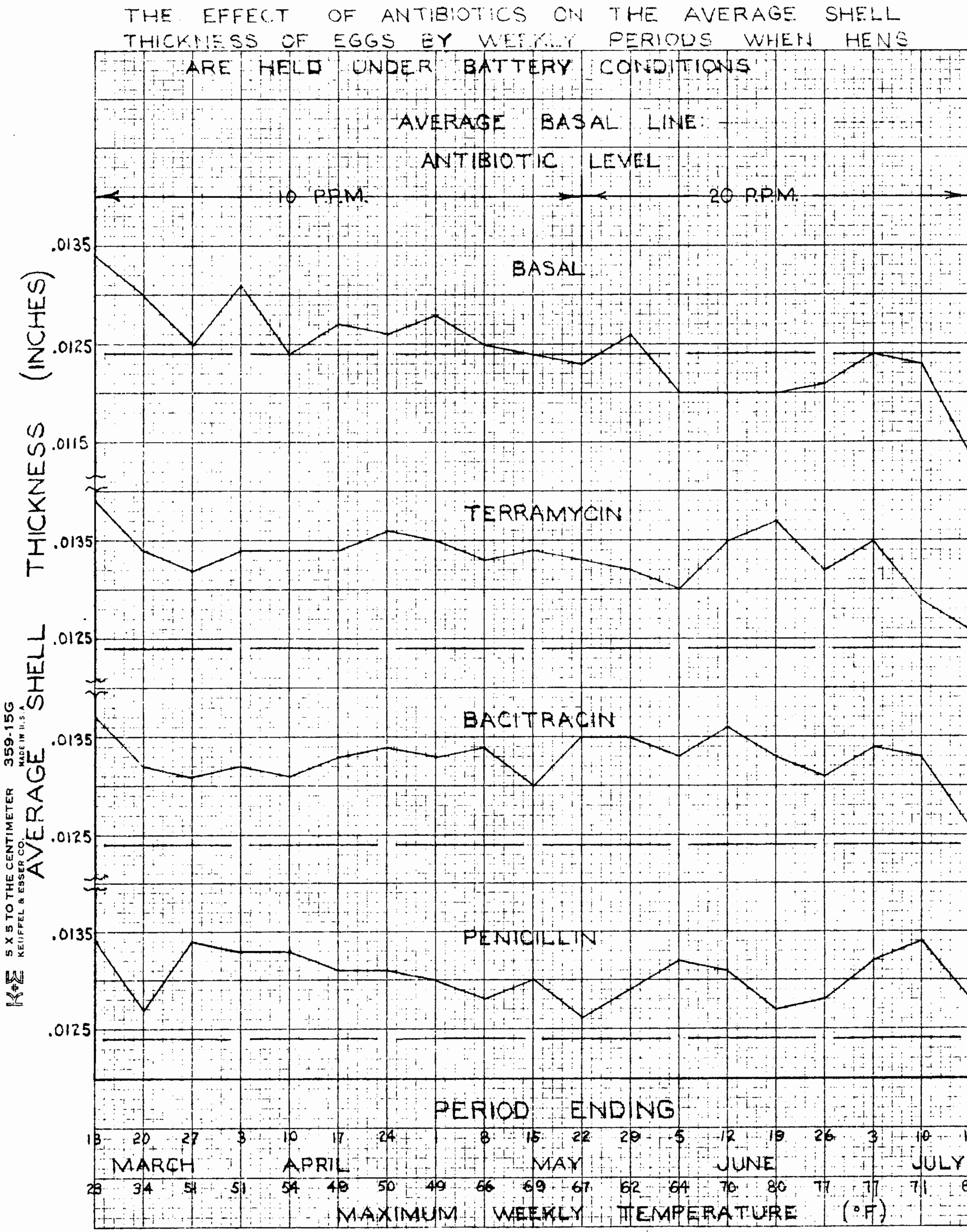
THE EFFECT OF ANTIBIOTICS ON THE AVERAGE SHELL THICKNESS OF EGGS BY WEEKLY PERIODS WHEN HENS ARE HELD UNDER BATTERY CONDITIONS
AVERAGE BASAL LINE
ANTIBIOTIC LEVEL
10 P.P.M.
20 P.P.M.
BASAL
TERRAMYCIN
BACITRACIN
PENICILLIN
AVERAGE SHELL THICKNESS (INCHES)
.0135
.0125
.0115
PERIOD ENDING
13 20 27 3 10 17 24 1 8 15 22 29 5 12 19 26 3 10 17
MARCH APRIL MAY JUNE JULY
28 34 51 51 54 49 50 49 66 69 67 62 64 70 80 77 77 71 83
MAXIMUM WEEKLY TEMPERATURE (°F)
K+E 5 X 5 TO THE CENTIMETER 359-15G
KEUFFEL & ESSER CO. MADE IN U.S.A.

FIG. 2.

Table 2. Blood calcium values of hens in batteries (in % of initial values) for the 10 p.p.m. and 20 p.p.m. level of antibiotic supplementation.

Treatment received.

	Penicillin			Bacitracin			Terramycin			Basal	
	10 p.p.m.	20 p.p.m.		10 p.p.m.	20 p.p.m.		10 p.p.m.	20 p.p.m.		10 p.p.m	20 p.p.m.
(4)[1]	86.3	91.7	(5)	96.6	105.8	(3)	102.2	110.4	(1)	103.6	76.3
(8)	109.4	105.8	(9)	107.5	101.6	(6)	124.4	132.0	(2)	99.6	85.1
(13)	101.8	98.6	(12)	93.7	101.4	(10)	100.4	99.1	(7)	102.8	78.3
(16)	137.0	130.1	(15)	116.9	101.4	(11)	102.3	117.1	(14)	98.4	86.8
Av.	108.62	106.56		103.67	102.55		107.32	115.4		101.1	81.62
Av. % change from initial	8.62	6.56		3.67	2.55		7.32	15.4		1.1	-18.38

[1]() - bird identity number.

D. Experiment 2

The effect of antibiotics on egg-shell quality in the laying hen under floor conditions of summer management.

Based on the early results obtained in Experiment 1, it seemed advisable to outline an experiment to observe the effect of the antibiotics under test on egg-shell quality, when fed to a larger group of birds held under normal conditions of floor management.

The design of Experiment 1 did not allow for a statistical treatment of the data because eggs cannot be obtained from each bird for each day, clutch periods vary from hen to hen and some hens will go off lay for an extended period, due to a partial moult, or to other uncontrollable factors. This data would be unavailable from each bird at specific daily periods. It has, however, been indicated that Experiment 1 was designed for preliminary observations of trends induced by the antibiotics being fed, and as such fulfilled its purpose. However, to remove some of these sources of variation and to allow for a statistical analysis of Experiment 2, the design outlined below was adopted.

(1) Experimental Plan

One hundred and twenty laying hens were selected from a flock of New Hampshire ~~and~~ X Barred Plymouth Rock crossbreeds. They were weighed and randomized into four pens, thirty hens to a pen. Wood shavings were used as the litter material. The four treatments which were assigned one to each pen at random, were as follows:

1. 50:50 laying mash to scratch grain and 20 ppm penicillin,

2. 50:50 laying mash to scratch and 20 ppm bacitracin,

3. 50:50 laying mash to scratch and 20 ppm terramycin,

4. 50:50 laying mash to scratch, not supplemented with antibiotic.

This level of antibiotic was fed until July 20, 1953 when the level was increased to 40 ppm in each of the three treatments 1, 2 and 3.

Feed, water, grit and shell were allowed ad libitum.

Ten birds were selected at random from each pen and were banded. Blood samples for calcium determinations were taken from the wing veins of these birds, at the beginning of the experiment, and at the end of the 20 p.p.m. period of treatment, and again at the end of the 40 p.p.m. period of treatment.

During the 10 p.p.m. period of supplementation ten eggs were selected at random in each pen from the daily production for the purpose of determining the specific gravity and thickness of shell. The specific gravity for the daily sample was determined at the end of each day. During the 40 p.p.m. period of supplementation a temporary drop in egg production occurred due to a partial moult and this necessitated the use of five eggs per pen per day rather than the ten eggs used in the earlier part of the experiment. Otherwise the plan followed in this portion of the experiment was the same as that followed when a level of 20 p.p.m. of antibiotics was fed.

With this design it was possible to obtain equal numbers of eggs per pen per day, thus exercising control over the variability involved when the production of individual birds was utilized as the statistical unit. The variation between days due to environmental, as well as to other uncontrollable factors, could then also be removed in the final analysis. Pen temperatures were recorded daily, but due to circumstances beyond control this data was destroyed. The temperatures recorded in the analysis of the data were obtained from the records kept at the Macdonald College weather recording station.

(2) Results and Discussion

The results obtained in Experiment 2 paralleled the trends already noted in Experiment 1. The average egg-shell thickness and specific gravity (Table 3) as the result of antibiotic feeding, were maintained at a higher level than in the control group.

Statistically an analysis of variance for both levels of antibiotic showed a highly significant difference between treatments for both egg-shell thickness and specific gravity. (See Appendix)

In the data recorded for the 20 p.p.m. level of supplementation, the least mean difference between treat-

Table 3. Average specific gravity and shell thickness of eggs from hens fed on antibiotic-supplemented rations and held under conditions of floor management. (Levels of antibiotic 10 p.p.m and 20 p.p.m.

Treatment	June 9 - June 20 (20 p.p.m.)		July 21 - August 18 (40 p.p.m.)	
	Specific gravity	Shell thick. (inches)	Specific gravity	Shell thick. (inches)
Penicillin	1.0746	.01318	1.0761	.01325
Bacitracin	1.0752	.01331	1.0767	.01349
Terramycin	1.0727	.01306	1.0754	.01312
Basal	1.0688	.01244	1.0725	.01251

ments required for significance in the case of the egg-shell thickness was 0.00008 inch. The mean values in Table 3 indicate that all the antibiotics induced greater thickness of shell when compared with the control. In addition they differed significantly within themselves in their ability to maintain this shell thickness at the higher level. In this respect bacitracin was superior to the other antibiotics, whereas penicillin was superior to terramycin.

Similarly, the least mean difference between treatments required for significance in the case of the specific gravity data was 0.0007. Here too there was a significant difference between the antibiotics in general and the control group with the specific gravity level in all cases being higher than the control group.

The superiority of bacitracin over penicillin, as indicated by the significant difference obtained between the mean of the egg-shell thicknesses of the two antibiotic treatments, was not reflected in the specific gravity data. The difference between the means of the two antibiotics was .0006 as compared with a required .0007 for significance at the 5% level.

For the 40 p.p.m. level of antibiotic feeding the data followed a similar pattern. Bacitracin and penicillin were, as in the case of the 20 p.p.m. level of supplementation, superior to terramycin in maintaining a higher level of both shell thickness and specific gravity. The least mean difference required for significance for shell thickness was .00007 inch and for specific gravity was .0007. The superiority of bacitracin over penicillin was again shown when the egg-shell thickness data were compared, but significant differences were lacking in the specific gravity data.

A graphical representation of the data appears in Figures 3 and 4.

An analysis of the correlation between average weekly temperature and average weekly egg-shell thickness for each of the three treatments yields the following correlation coefficients:

1) Penicillin r - -.406

2) Bacitracin r - -.58

3) Terramycin r - -.41

4) Basal r - -.54

These correlation coefficients were non-significant at the 5% level. The negative correlation trend however, seems to be present and, in general, egg-shell thickness seems to deteriorate as temperature rises. The fact that there is a lack of significance in the correlation coefficients may have been due to one or both of two factors:

a) The temperatures used in the analysis were outside temperatures and may not have corresponded to the actual conditions within the pens.

b) The correlation involved here may have been a serial correlation in which the response of egg-shell thickness to temperatures would have been dependent on prolonged periods of either high temperatures or low temperatures, that is, to

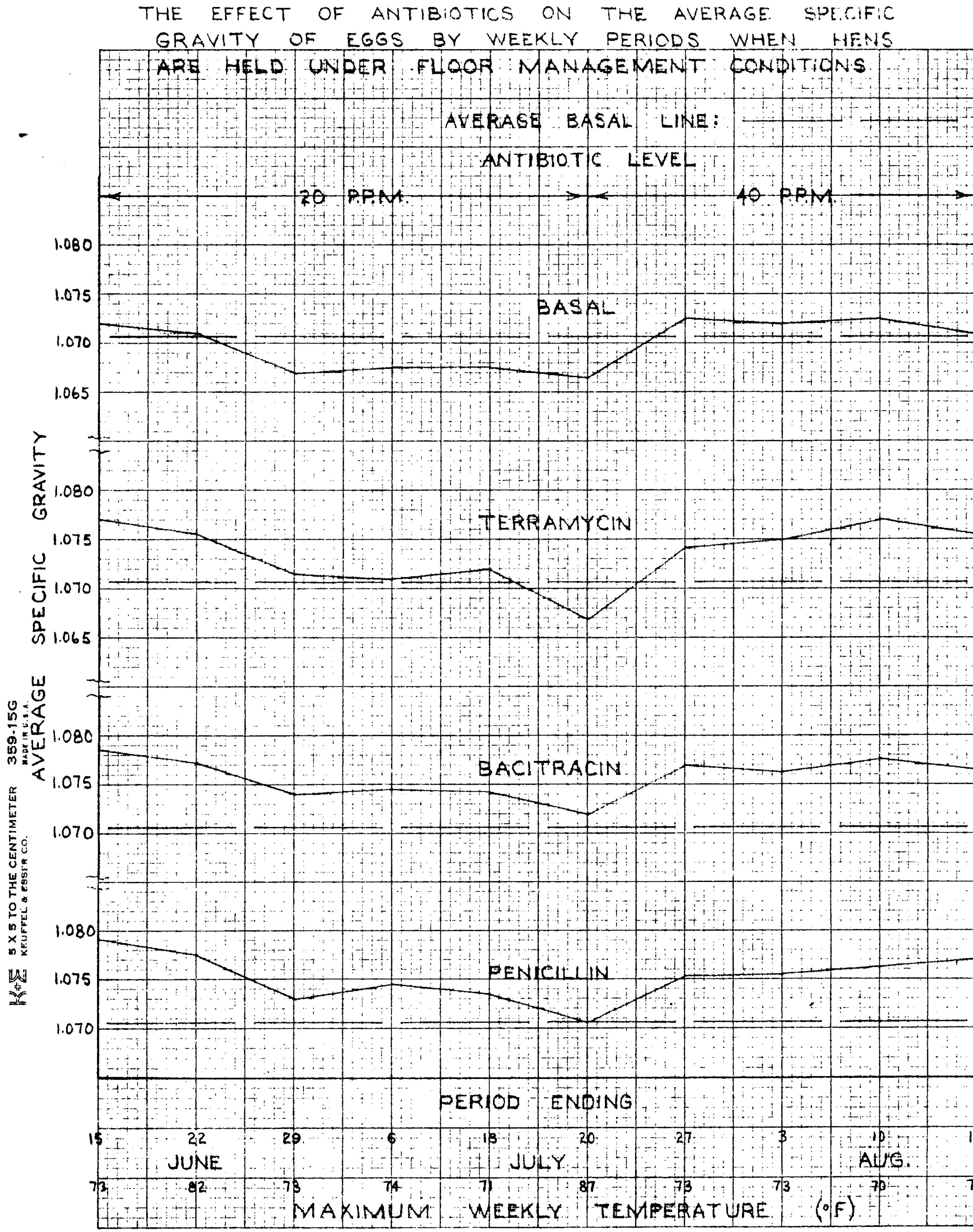
THE EFFECT OF ANTIBIOTICS ON THE AVERAGE SPECIFIC GRAVITY OF EGGS BY WEEKLY PERIODS WHEN HENS ARE HELD UNDER FLOOR MANAGEMENT CONDITIONS
AVERAGE BASAL LINE:
ANTIBIOTIC LEVEL
20 P.P.M.
40 P.P.M.
BASAL
TERRAMYCIN
BACITRACIN
PENICILLIN
1.080
1.075
1.070
1.065
AVERAGE SPECIFIC GRAVITY
PERIOD ENDING
15 22 29 6 13 20 27 3 10 18
JUNE
JULY
AUG.
73 82 73 74 71 87 73 73 70 78
MAXIMUM WEEKLY TEMPERATURE (°F)
359-15G
MADE IN U.S.A.
5 X 5 TO THE CENTIMETER
KEUFFEL & ESSER CO.

FIG. 3

THE EFFECT OF ANTIBIOTICS ON THE AVERAGE SHELL THICKNESS OF EGGS BY WEEKLY PERIODS WHEN HENS ARE HELD UNDER FLOOR MANAGEMENT CONDITIONS

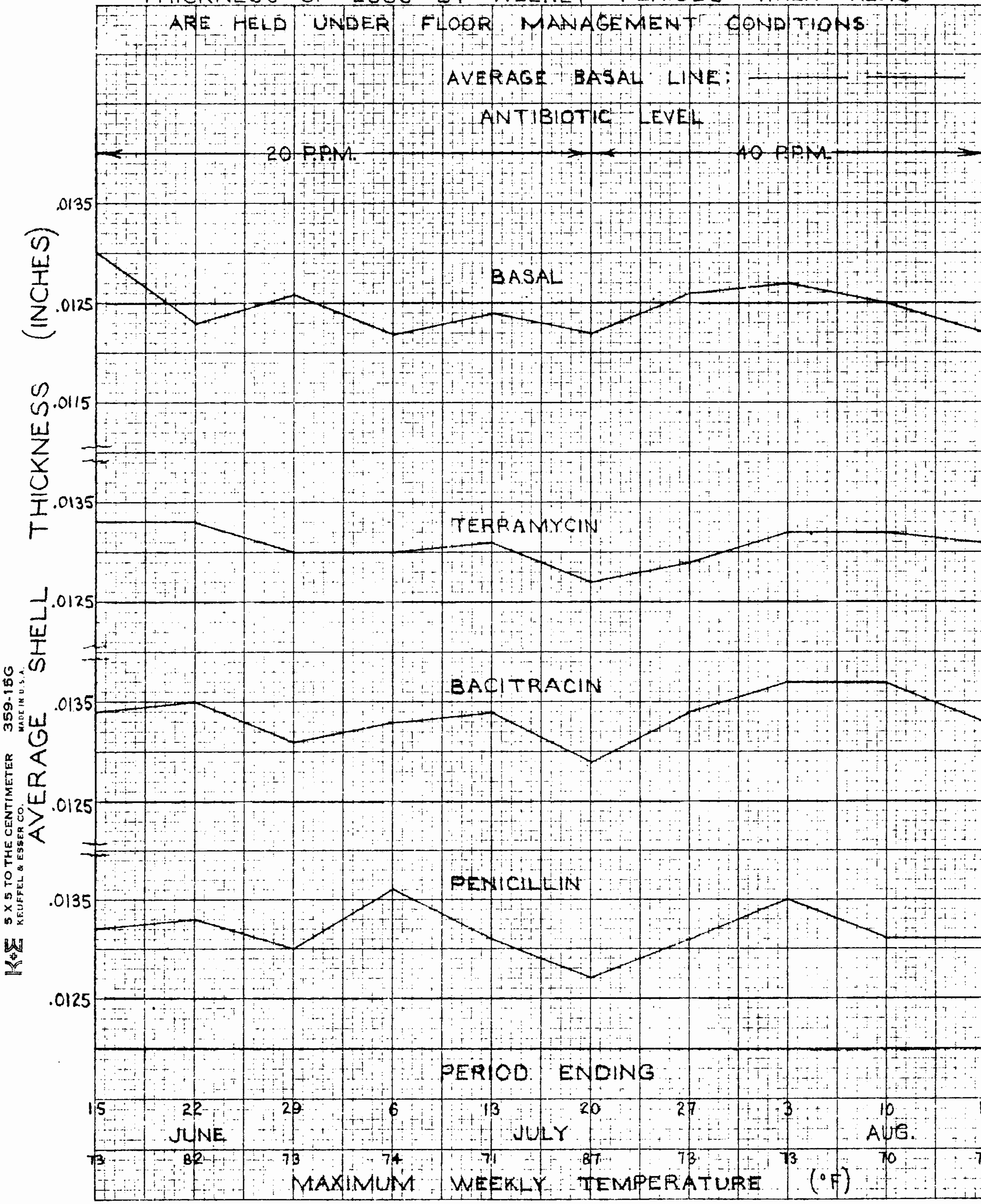

FIG. 4

reduce the egg-shell thickness by .001 inch it may have been necessary for the high temperatures to have prevailed over a period of more than one day.

The weekly temperatures used in the analysis merely reflected the average conditions for the week, and two or three high temperature recordings for the week would have contributed to a high average value, but the duration of the period of high temperatures during the week may not have been sufficient to cause the lowering in shell thickness.

The experimental plan followed here did not allow for a test of serial correlation as individual bird data would have had to be utilized for such an analysis.

The specific gravity results correlated with temperature followed the same trends. The correlation coefficients were non-significant, but the negative correlation trend was also noted here.

If the hypothesis of a possible serial correlation as advanced in the case of the egg-shell thickness data is correct, the same conditions would have applied here, as the correlation between specific gravity and egg-shell thickness was significant at the 5% level in both the case of the 20 p.p.m. period of antibiotic supplementation and the 40 p.p.m. period.

Table 4 demonstrates the effect ~~observed~~ of the antibiotics on the level of blood calcium under the two levels of supplementation. The level of blood serum

Table 4. Blood calcium values (in % of initial values) of hens on floor for the 20 p.p.m. and 40 p.p.m. levels of antibiotic supplementation.

	Antibiotic Fed										
	Penicillin			Bacitracin			Terramycin			Basal	
	20 p.p.m.	40 p.p.m.		20 p.p.m.	40 p.p.m.		20 p.p.m.	40 p.p.m.		20 p.p.m.	40 p.p.m.
(1)	82.6	100.0	(11)	103.3	114.4	(21)	100.4	105.4	(31)	102.0	99.0
(2)	107.9	104.0	(12)	122.6	125.6	(22)	93.6	91.9	(32)	89.9	72.2
(3)	107.5	115.6	(13)	104.3	108.6	(23)	81.7	85.6	(33)	104.3	94.6
(4)	135.8	118.4	(14)	93.9	82.7	(24)	104.3	102.7	(34)	87.5	102.0
(5)	117.7	104.3	(15)	100.9	106.9	(25)	109.9	105.2	(35)	87.3	87.0
(6)	85.9	84.6	(16)	110.9	87.9	(26)	85.8	95.3	(36)	100.5	107.0
(7)	100.4	111.9	(17)	99.0	98.0	(27)	124.8	.26.0	(37)	83.7	87.5
(8)	100.5	100.0	(18)	102.2	100.7	(28)	95.6	92.4	(38)	106.0	100.0
(9)	86.9	92.6	(19)	99.6	110.9	(29)	82.3	90.4	(39)	96.1	96.5
(10)	112.6	106.1	(20)	97.0	95.0	(30)	100.7	98.3	(40)	99.0	92.6
Average %	103.78	103.75		103.37	103.07		97.91	99.32		95.63	93.84
Av. % Change	3.78	3.75		3.37	3.07		-2.09	-0.68		-4.37	-6.1

() Bird identity number

calcium under penicillin and bacitracin feeding was statistically significantly superior in both cases to the blood serum calcium level of the control group. The mean difference between the terramycin group and the control was not sufficient to register a significant difference in the calcium level. Penicillin and bacitracin appeared to possess equal value in maintaining a higher level of serum calcium.

E. Experiment 3

(a) The effect of antibiotics in bone calcification in chicks when included in a vitamin D_3 deficient ration

The previous experiments had been devoted to a study of the actual effect of the antibiotics under investigation on egg-shell quality. In contrast, this phase of the work was designed to study the possible mechanisms involved in the apparent mobilization of calcium previously discussed.

In this series of experiments, therefore, the plan was to investigate the relationship between the antibiotics and three of the factors known to be most actively associated with calcification, i.e., levels of vitamin D_3, calcium and phosphorous.

A logical hypothesis was to assume that the antibiotics in some way increased the efficiency of utilization of either vitamin D_3, calcium, ~~or~~ phosphorous or all of them, and to follow a plan that would allow for a test of this assumption.

The basis of the plan was to feed rations deficient in the nutritional elements under study and to observe the effect of the antibiotic supplements under such conditions. If the assumption was justified there should be an observable difference in calcium metabolism in favour of the antibiotic-supplemented groups, even under conditions of a deficiency of the nutritional elements involved.

The first experiments in this series, Experiments 3(a) and 3(b), involved a determination of the effect of the three antibiotics upon calcification in chicks when a diet containing sub-optimal amounts of vitamin D_3 was fed.

(1) Experimental Plan

New Hampshire X ~~and~~ Barred Rock male and female one-day old chicks were used in this experiment. Sixty-eight one-day old chicks were randomized into four groups of 17 chicks per group. The chicks were weighed, banded, and placed at random in a battery brooder. The chicks were weighed at weekly intervals while on test.

The treatment consisted of a modified A.O.A.C. rachitogenic diet divided into 4 lots, A, B, C and D. (See Appendix for composition of ration)

The lots A, B, C and D were supplemented as follows:

A. Basal, no antibiotic

B. Basal - bacitracin (Commercial Solvents) 10 p.p.m.

C. Basal - terramycin hydrochloride (Pfizer) 10 p.p.m.

D. Basal - penicillin (Merck) 10 p.p.m.

The dietary treatments A,B,C and D were assigned at random to the groups of chicks.

A standardization period of 3 days was allowed during which time approximately 12 oz. of the respective feeds ~~was~~ were placed in each hopper. At the end of this period, the basal group was allowed feed ad libitum but the birds on the antibiotic treatments were restricted to the average daily consumption of the chicks on the basal diet. The average daily consumption of the basal group was determined by placing 14 oz. of feed in the hopper and obtaining feed intake by difference at the same time on the next day. Water was allowed ad libitum to all groups. At the end of 3 weeks the chicks were killed and the tibia of the left leg removed for (percentage) ash determinations.

During the experiment, it was observed that a number of the chicks were down on their hocks in numbers varying with the imposed treatment, and as a result dates of the occurrence of this abnormal condition were taken and separate records were kept of the ash content of lame birds in the analysis of the results.

(2) Results and Discussion

The average differences in tibia ash under the four treatments have been recorded in Table 5. Analysis of the data yielded results which indicated a non-significant difference between the treatments under the conditions of this experiment. The variation within the treatment groups was large and probably reduced the effectiveness of the test between treatments. An indication of this variation has been illustrated by the range of ash values included in Table 5. From these values it can be seen that, at least in the case of the group receiving the penicillin-supplemented feed, the upper range of percent ash value was higher than in the other treatments.

However, in general, under the conditions of this experiment it appeared that the antibiotics themselves were

Table 5

Summary average weight gains and percent tibia ash at end of three-week period for the various groups of chickens receiving different treatments.

Group and treatment		Average live weight gain	Average % ash	Range of ash Low	High
A.	Basal	69.6	34.1	31.4	39.2
B.	Bacitracin	56.7	34.6	31.2	38.6
C.	Terramycin	65.1	35.0	31.3	38.6
D.	Penicillin	59.7	35.6	32.4	47.1

not active in any way in increasing calcification in the absence of vitamin D_3.

It may be recalled here that in the experiment conducted by Lindblad et al.(23), as noted in the literature review, increased calcification was obtained when vitamin D_3 was included in an antibiotic-supplemented ration, so that the role of the antibiotic may have been to induce an increase in the efficiency of utilization of the vitamin D_3 and not in the replacement of vitamin D_3 as a necessary constituent of the calcification process. In the experiment referred to, the reason for the efficiency of the antibiotic in this respect at the 8 I.U. and 30 I.U. levels of vitamin D_3 fed, and not at the 4 I.U. and 600 I.U. levels may have been due, in the case of the lower level, to an absolute insufficiency of vitamin D_3 and consequently to a possible free uptake of all the available vitamin. In the case of the higher levels of vitamin D_3, the vitamin was present in more than adequate amounts, and the requirements of the vitamin may have been fully met, so that any effect of the antibiotic would have been masked under these conditions.

The weight gains during the period of feeding appear in Table 5. Here too, there was a lack of a significant difference between treatments.

It may be noted that similar results were obtained by Scott and Glista (39) under paralleled conditions of feed management, when the feed of antibiotic-supplemented birds was limited to the intake of a control group. Part of the effect of antibiotics in causing weight increases, as reported by numerous workers including Stokstad and Jukes (43), Scott and Glista (39), etc., may have been due to an accompanying increased feed consumption induced by an appetite stimulated by antibiotic supplementation.

Experiment 3

(b) The effect of antibiotics on bone calcification at weekly intervals when included in a vitamin D_3 deficient ration.

During the course of Experiment 3(a), it was observed that the lameness associated with vitamin D_3 deficiency symptoms was followed, in some cases, by a period of partial recovery in which the lameness seemed to have been absent or less severe.

It was, therefore, thought desirable to design an experiment to test the possibility of a relationship between the antibiotics and bone calcification over different time intervals.

(1) Experimental Plan

The experimental plan adopted here was essentially the same as in Experiment 3(a).

Seventy-two chicks were randomized into four groups of 18 chicks per group to correspond to the four treatments. Each group was further divided into two lots of 9 chicks per lot to provide a replicate of the experiment. Treatment and management conditions were the same as in Experiment 3(a).

At the end of each of the three weeks of the experimental period three chicks per replicate were sacrificed. The tibia of the left leg was removed as in Experiment 3(a) for the purpose of percent bone ash determinations. Weekly weight gains were also recorded.

(2) Results and Discussion

The overall differences between treatments for both bone ash and weight gains, were similar to the results obtained in Experiment 3(a) in that the differences existing between treatments were non-significant. (Tables 6 and 7).

A significant difference occurred between weekly periods for the percent bone ash data. The second week appeared to be the most critical in the case of the basal and bacitracin groups and percent bone ash for these treatments was at its lowest during that period. This factor probably accounted for the apparent partial recovery from lameness observed in some cases in Experiment 3(a). In the other two treatments, penicillin and terramycin, the lowest percent bone ash values were noted during the third week of the treatment.

As was to be expected, a progressive average increase in weight gains was recorded from the end of the first week to the end of the second and third weeks respectively. The average increase was greatest in the case of the terramycin-supplemented group at the end of the third week, and smallest in the case of the bacitracin-supplemented group. A comparison of Experiments 3(a) and 3(b) indicated that the average percent bone ash at the end of the first experiment was, on the average, greater than the percent bone ash during a similar period in the second experiment. In this respect, it is to be noted that New Hampshire chicks were used in the second experiment as compared with a New Hampshire X Barred

Table 6. Average of weight gains im gms. during the three weekly periods for chicks receiving different treatments.

Treatment		Average weight gain at end of week 1	2	3
A.	Basal	34.3	48.0	60.0
B.	Bacitracin	31.0	44.8	55.8
C.	Terramycin	33.2	56.0	65.0
D.	Penicillin	28.2	38.5	63.0

L.S.D. for mean difference between treatments for 1 weekly period - 18.7

Table 7. Average percent tibia ash during the three weekly periods for chicks receiving different treatments.

Treatment		Average percent tibia ash at end of week 1	2	3
A.	Basal	35.5	30.3	32.2
B.	Bacitracin	32.5	30.3	31.0
C.	Terramycin	34.1	32.0	29.8
D.	Penicillin	37.8	34.0	28.5

L.S.D. for mean difference between treatments for 1 weekly period - 13.4.

Rock cross in the first. This factor could in some measure have contributed to the difference observed in the case of the two experiments.

From Experiment 3(a) and 3(b) it appeared that the antibiotics were not active in influencing calcification in the complete absence of supplementary sources of vitamin D_3. It appeared then, that the role of the antibiotics, as far as their relationship to calcification and vitamin D_3 was concerned, was not associated with replacement of vitamin D_3. The possibility of an increased absorption of vitamin D_3 under antibiotic stimulation, as recorded by Lindblad and others, may have been responsible for the differences observed by them.

F. Experiment 4

The effect of antibiotics on bone calcification when included in diets (i) low in calcium and (ii) low in phosphorous.

It has been established that, besides vitamin D_3, the other factors involved in calcium metabolism are the minerals phosphorous and calcium. An experiment was therefore designed to test for possible association between these two minerals and the antibiotics.

The basic plan was similar to that followed in the preceding experiment, in that antibiotic~~s~~ supplements were added to rations deficient in calcium but adequate in phosphorous and vitamin D_3 on the one hand, and deficient in phosphorous but adequate in calcium and vitamin D_3 on the other. The object was to test for difference in bone calcification due to the antibiotic supplements.

(1) Experimental Plan

Seventy-two day-old New Hampshire ~~and~~ X Barred Rock chicks were randomized into four groups of 18 chicks per group and placed in four separate compartments of a battery brooder. One of the four treatments viz. basal, basal plus penicillin, basal plus bacitracin and basal plus terramycin were assigned to one of the four groups at random. By the use of a dividing partition, each group corresponding to one of the treatments was sub-divided into two sub-groups: a low-calcium sub-group, and a low-phosphorous sub-group of nine chicks per sub-group. The chicks were banded , and to allow for detection of a possible differential effect of antibiotic at different intervals during the course of the experiment, the chicks in each sub-group were chosen at random in sample lots of 3 chicks per lot to be sacrificed for bone ash determinations at the end of one week, two weeks and three weeks respectively.

The experiment was replicated so that a total of 144 chicks, 72 chicks per replication, were used. A plan of the experimental design appears in Figure 5.

The approximate content of phosphorous and calcium in the basal diet was .69% for phosphorous and .35% for calcium.

The diet to be utilized for the low-calcium study was supplemented with 2 lb. of duo hydrated Sodium Phosphate, and in the case of the low-phosphorous diet, 2 lbs. ground lime-stone was added to the basal ration. This resulted in a total content of .35% calcium and 1.03% phosphorous in the low-calcium ration, and .69% phosphorous and 1.11% calcium in the low-phosphorous diet.

The low-calcium and low-phosphorous diets were supplement-ed with antibiotics as follows:

(See over)

Fig. 5. Design of experimental plan for antibiotic-supplemented low calcium and low phosphorous experiment.

		Compartment 1 Basal		Compartment 2 Penicillin		Compartment 3 Bacitracin		Compartment 4 Terramycin	
		Low ca	Low ph	Low ca	Low ph	Low ca	Low ph	Low ca	Low ph.
		Weeks		Weeks		Weeks		Weeks	
		1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3
Rep.	S1.								
I	S2								
	S3								
Rep.	S13								
II	S14								
	S15								

S : Sample

Treatment	Diet
A_1	Basal low calcium and no antibiotic.
A_2	Basal low phosphorous and no antibiotic.
B_1	Basal low calcium plus 10 p.p.m. bacitracin.
B_2	Basal low phosphorous plus 10 p.p.m. bacitracin.
C_1	Basal low calcium plus 10 p.p.m. terramycin.
C_2	Basal low phosphorous plus 10 p.p.m. terramycin.
D_1	Basal low calcium plus 10 p.p.m. penicillin.
D_2	Basal low phosphorous plus 10 p.p.m. penicillin.

Management conditions were as described in Experiments 3(a) and 3(b). Water was allowed ad libitum and feed was restricted to the intake of the basal lot.

At the end of each weekly period until the end of the third week, 3 chicks were sacrificed from each sub-sub-group for bone ash determinations. Weight gains were recorded at the end of each week.

(2) Results and Discussion

Due to unavoidable circumstances, the tibias obtained from the bacitracin-supplemented group were destroyed so that the analysis and discussion of the bone ash data is limited to the treatments - basal control, terramycin and penicillin.

The average percent bone ash over the experimental period (including both low calcium and low phosphorous) were as follows:

Basal -------- 36.4%

Bacitracin --- 36.6%

Terramycin --- 36.6%

These differences were small and not significant.

The critical level of percent bone ash, which was apparent during the second weekly interval of the low vitamin D_3 experiment, was not recorded in the case of the present experiment.

The differences due to mineral treatment were significant, the chicks on the low-phosphorous ration exhibiting a lower average percent bone ash than the chicks on the low-calcium diet.

The interaction of antibiotic between low calcium and low phosphorous showed no significant differences. (See Appendix)

The weight gain data recorded in Table ~~11~~ 8 differed from the bone ash data in Table ~~10~~ 9 in several aspects:

i) A significant difference was obtained between main treatment groups (basal, bacitracin and terramycin), with the antibiotics penicillin and terramycin giving more satisfactory weight gain increases than the basal or bacitracin-supplemented groups.

ii) Differences between weekly periods were significant.

iii) Greater weight gain increases were obtained on antibiotic-supplemented, low-calcium diets than on the basal low-calcium diet.

With reference to (i), it has been noted that antibiotics have been found to increase weight gains under some

Table 8. Average weight gain in gms. of chicks on low calcium and low phosphorous anti-biotic-supplemented rations.

Weeks	Basal		Penicillin		Bacitracin		Terramycin	
	Low cal.	Low phos.	Low cal.	Low phos.	low cal.	low phos.	low cal.	low phos.
1	24.6	24.0	25.7	26.0	23.3	17.0	24.7	25.3
2.2.	57.8	44.2	77.1	39.8	44.7	26.2	56.7	57.1
3.	71.3	58.7	125.1	83.7	113.0	79.3	124.8	90.7

Table 9. Average percent bone ash of chicks on low calcium and low phosphorous antibiotic-supplemented rations.

Weeks	Basal		Penicillin		Terramycin	
	Low cal.	Low phos.	Low cal.	Low phos.	Low cal.	Low phos.
1.	39.3	34.3	41.1	33.0	39.2	30.5
2.	38.6	30.5	41.3	30.8	41.8	32.0
3.	41.3	34.5	42.1	31.6	43.1	34.5

experimental conditions. The fact that this effect was not observed in the previous experiments has been ~~could be~~ attributed to the limitation of the feed intake of the supplemented groups to that of the basal groups. The probability that this factor was only in part responsible for the observed results was borne out in this experiment, where despite the similar conditions of feed limitation, the penicillin and terramycin-supplemented groups have given evidence of increased weight gains, when compared to the basal lot. The bacitracin-supplemented group did not differ significantly from the basal group although, on an average basis, a greater average weight gain was recorded for the antibiotic-supplemented group.

In the case of (ii), it would be expected that differences in gain over different periods of time would be significant and the results obtained here only served to illustrate that the normal pattern was followed in this experiment.

Whereas no significant effect of interaction between main group and sub-group treatments was recorded, the chicks receiving antibiotic-supplemented, low-calcium rations showed greater weight gains than the chicks not receiving the supplement. In this respect, the average differences between the antibiotics, terramycin and penicillin, and the basal group was significant. The bacitracin-supplemented group, while exhibiting the same trend, did not differ significantly from the basal group. The differences between penicillin and terramycin were not significant. (See Appendix)

It was evident then, that even under conditions of a low level of calcium in the ration, the antibiotics were able to influence the growth rate significantly.

In the light of the evidence obtained from the weight-gain data two assumptions can be made:

i) that in some manner the antibiotic was able to replace part of the calcium requirement;

ii) that the antibiotics were able to increase the efficiency of utilization of the calcium present in the ration.

The first assumption can be disregarded as it is highly unlikely that the presence of antibiotics can replace the calcium requirements.

The question of an increased efficiency of calcium utilization therefore seems to be the only point of concern. Any attempt to explain the mechanism which could be involved in the increased utilization of calcium under antibiotic stimulation can, at present, only be speculative.

Recent work reported by Sieburth et al (40) suggests one avenue by which increased absorption of calcium could take place under antibiotic stimulation. They demonstrated a distention of the intestinal blood vessels in the hen under conditions of antibiotic feeding. With the consequently increased flow of blood from the intestine it is likely that an increase in calcium absorption could take place. It would be expected that, if such were the case, the efficiency of nutrients other than calcium would be increased when incorporated in an antibiotic supplemented ration. Evidence in that direction at present is not consistent. Biely et al (9) showed an

increase

in feed efficiency when an aureomycin fermentation product was included in a 17% protein diet containing fish meal. Berg et al. (5) obtained no differences in feed conversion rates when this product was added to a diet containing fish meal. Carpenter (10) demonstrated an increase in feed efficiency when crystalline aureomycin was added to diets containing vitamin B_{12}.

Results obtained by other workers have varied with the experimental conditions, and no consistency in response has been established. However, the fact that in some cases the pattern of response has tended towards an increase in feed utilization in response to antibiotic stimulation would seem to indicate that, at least in these cases, the mechanism involved may be connected with an increase in the efficiency of absorption of the nutrients under study.

With reference to the differences observed for the low-calcium and low-phosphorous diets, the following factors are to be noted.

Evidence gathered by Ewing (17) from several workers indicated that the minimum requirements of calcium for growing chicks was about 0.70 percent and that of phosphorous 0.40%.

The phosphorous present in the basal ration fed was .68% whereas the calcium level was .35%. However, 70% of the phosphorous was from plant sources, in which a substantial part of the phosphorous present is bound in the phytin-molecule, a form in which it is relatively unavailable to the chick. The level of phosphorous, as calculated for the low-phosphorous ration, was therefore not a true indication of the amount of phosphorous which was actually available for metabolic processes. In contrast the calcium present could readily be utilized. Thus in both the low-calcium and low-phosphorous diets approximately a half of the normal requirements was present in the basal ingredients of the ration.

Under these conditions it appeared that the requirement of phosphorous in the presence of adequate amounts of calcium was more critical than the requirement of calcium in the presence of adequate amounts of phosphorous. As a result, both in the percent bone ash data and the data recorded for gain in weight, consistent differences in favour of the low-calcium ration were obtained.

G. Experiment 5

The effect of antibiotics on egg-shell quality when levels of calcium intake are controlled.

In an attempt to study the relationship between low levels of calcium and the presence of antibiotics in the hens' ration, an experiment was conducted in which regulated amounts of calcium, in the form of oyster shell, were fed to hens receiving antibiotic supplements. Specific gravity and shell thickness data, as criteria of response, were recorded for the period of the experiment.

(1) Experimental Plan

This experiment was divided into two parts:

i) A study of the effect of the antibiotics on egg-shell quality on a minimum calcium diet when the environmental temperature was allowed to fluctuate with outside weather conditions;

ii) A study of the same diet when temperature conditions were held at a constant high level.

The general plan for both experiments was as follows:

Sixteen New Hampshire X Barred Rock hens were placed at random, one hen in each compartment of a laying battery. The four treatments - basal, bacitracin, penicillin and terramycin - were assigned at random to the hens so that there were four hens per treatment.

The ration fed was the 50:50 laying mash to scratch, fed in the previous experiments conducted under battery and floor conditions, and the level of the antibiotic incorporated into the diet was 20 p.p.m. Feed, water and grit were allowed ad libitum.

One gram of oyster shell was administered orally each day by placing the crushed shell in a gelatin capsule.

During the first section of the experiment the hens were kept in quarters in which the temperature varied with outside weather conditions. This was conducted from November 24 to January 4, 1953.

During the second part of the experiment the temperature of the room was raised to approximately 75°F and held at that temperature throughout the experimental period.

Specific gravity and egg-shell thickness measurements were recorded during both experiments.

(2) Results and Discussion

The summary of the results during the first part of the experiment is recorded in Table 10.

It would appear from the data obtained that, with the exception of the hens allowed the penicillin supplement, the antibiotics in general failed to maintain a higher level of shell thickness and specific gravity when compared to the control group. The slightly lower specific gravity recorded in the control group was caused by the relatively low mean value recorded for bird number 15. In the penicillin-supplemented group both the average specific gravity and shell thickness measurements were somewhat higher than the basal group and likewise terramycin and bacitracin-supplemented groups.

The average temperature during this period of the

Table 10. Average specific gravity and shell thickness of eggs on minimal calcium diet under low temperature conditions.

Treatment	Bird No.	Average Sp. Gr.	Average shell thickness (ins.)	Average for treatment. Sp. Grav.	Shell thi.
Penicillin	1	1.0758	.0118		
	2	1.0775	.0126		
	3.	1.0759	.0123		
	4.	1.0756	.0123	1.0762	.0123
Bacitracin	5.	1.0720	.0100		
	6.	1.0738	.0115		
	7.	1.0750	.0120		
	8.	1.0773	.0126	1.0745	.0115
Terramycin	9.	1.0749	.0122		
	10.	1.0730	.0110		
	11.	1.0735	.0112		
	12.	1.0749	.0116	1.0741	.0115
Basal	13.	1.0739	.0112		
	14.	1.0740	.0120		
	15.	1.0690	.0107		
	16.	1.0750	.0118	1.0731	.0114

Average Temperature 50° F.

experiment was 50° F. It has already been demonstrated that high temperatures, as encountered under summer conditions, will cause a deterioration in egg shell and specific gravity.

In the absence of this stress factor it appeared that the antibiotics in general were unable to influence calcification on a low-calcium diet.

The second part of the experiment was designed to s~~t~~imulate the temperature stress factor encountered under summer conditions and to test the effect of the antibiotics under low-calcium intake.

The results (Table 11) indicated that under the imposed experimental conditions the antibiotics, with the exception of penicillin, did not seem to have an effect on either shell thickness or specific gravity although a general decline from the first experimental period in both these physical properties was induced, presumably by the increased temperatures.

Failure to reproduce the results obtained in the previous experiments conducted under normal conditions of summer management could be due to a number of causes.

One such cause may be that, in an effort to duplicate summer conditions, artificial temperature conditions were imposed. The relationship between shell deterioration and summer temperature conditions may, however, extend further than the relationship due to increased temperatures; ~~and~~ other inter-related factors encountered under summer conditions, may contribute to the lowering of egg-shell thickness and specific gravity. In such a case the disturbance of the

Table 11. Average specific gravity and egg-shell thickness on minimal calcium diet under high temperature conditions.

Treatment	Bird No.	Average Sp. Gr.	Average shell thickness	Average for treatment Sp. Grav.	Shell thick.
Penicillin	1	1.0720	.0115		
	2.	1.0742	10122		
	3.	1.0736	.0120		
	4.	1.0730	.0118	1.0732	.0119
Bacitracin	5.	1.0695	.0100		
	6.	1.0700	.0110		
	7.	1.0732	.0116		
	8.	1.0740	.0120	1.0717	.0112
Terramycin	9.	1.0729	.0118		
	10.	1.0672	.0108		
	11.	1.0710	.0110		
	12.	1.0725	.0114	1.0709	.0113
Basal	13.	1.0698	.0110		
	14.	1.0732	.0118		
	15.	1.0668	.0105		
	16.	1.0730	.0116	1.0707	.0112

Average Temperature 70 F.

physiological mechanisms which contribute to the deterioration in egg-shell quality may not have been totally accomplished under the imposed experimental conditions. The reduction in egg-shell thickness and specific gravity noted ~~then~~ could only be attributed to the limited intake of calcium on the one hand and to the relationship due to increased temperatures on the other.

The fact that improvement in shell quality was obtained only in feeding the penicillin-supplemented diet would seem to indicate that, although in general, the antibiotics have been found to influence calcification under conditions of summer management, the path of action may not be the same for all antibiotics. The dilation of the intestinal blood vessels, as previously discussed, ~~have~~ has to date only been related to the presence of penicillin in the diet and no work has been reported on the other antibiotics used in the experiment.

Under limited intakes of nutrients, the efficiency of utilization of any specific nutrient is known to increase, so that during the low-calcium intake, as imposed in this experiment, the utilization of the calcium present could have already been at a maximum and the antibiotics were unable to further influence calcium absorption.

III SUMMARY

The economic problem coincident with the deterioration of egg-shell quality under summer management conditions has prompted an investigation of reported effects of antibiotics on calcium metabolism.

This study was divided into two phases. The first phase involved a determination of the effect of the antibiotics bacitracin, penicillin and terramycin on egg-shell thickness and the specific gravity of eggs, obtained from hens receiving these antibiotics in the laying ration. The results from experiments conducted in this investigation have led to the following conclusions:

(1) The decline in egg-shell thickness and specific gravity of eggs, which is associated with the onset of summer conditions, was minimized by the inclusion of the antibiotics under test in the laying ration. In this respect bacitracin and penicillin were more consistent than terramycin.

(2) Blood calcium levels were maintained at a higher level in the presence of the antibiotics in the laying ration.

The second phase of the study was undertaken on the basis of the evidence obtained in the first phase of the investigation. Possible mechanisms involved in the observed relationship of antibiotics to increased shell deposition were studied. Experiments designed to test for possible relationship between the antibiotics and (i) vitamin D_3, (ii) calcium and (iii) phosphorous, yielded the following results:

(i) In the absence of ~~supplemented~~ vitamin D_3 in the ration of chicks, the antibiotics were unable to cause an increase in bone calcification.

(ii) Similarly the antibiotics were unable to influence calcification on a chick diet low in phosphorous.

(iii) Whereas no significant differences in bone calcification were obtained on antibiotic-supplemented low-calcium diets when compared with a control group, weight gain differences on the antibiotic-supplemented low-calcium diet were significantly greater than the weight gains recorded for the low-calcium, non-supplemented group. This suggested an increased efficiency of utilization of the calcium present in the ration, when the antibiotics were included. The effect of penicillin was the most pronounced in this respect.

Further studies were made on the effect of the antibiotics in the rations of laying hens receiving sub-optimal amounts of calcium. No relationship was established under the experimental conditions imposed. Subjecting the hens to high temperature conditions and low calcium intake resulted in decreased egg-shell thickness and specific gravity of eggs. The condition was not improved by the inclusion of antibiotics in the ration. The results recorded here indicated that the effect of temperature was only in part responsible for the decrease in egg-shell quality encountered under summer conditions of management.

As only one level of calcium was fed in this experiment, the results obtained are not conclusive and it is desirable to test the antibiotics in association with different dietary levels of calcium before a final decision can be made.

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V. APPENDIX

Composition of basal rachitogenic diet for vitamin D_3 deficient experiment.

Ground Barley	28 lb.
Wheat middlings	25 "
Soybean meal	6 "
Fish meal	6 "
Dried brewers' yeast	2 "
Rock phosphate	2 "
Salt	1 "
$MnSo_4$	.01 %
Vitamin A supplement Napco dry form 5000 IU/gram.	60 gms.

Composition of basal ration for low calcium and low phosphorous experiments.

Ground barley	28 lbs.
Wheat middlings	25 "
Soya bean meal	6 "
Fish meal	6 "
Dried brewers' yeast	2 "
Salt	1 "
$MnSo_4$	.01 %
Vitamin A Napco dry form 5000 IU/gram	60 gms.
Vitamin D_3	9000 i c u

2 lbs. duo hydrated sodium phosphate added to the above ration for calcium deficient ration and 2 lb. of ground limestone for the phosphorous deficient ration.

Experimental Laying Mash

Ground wheat	200lbs.
Ground barley	200 "
Ground oats	75 "
Wheat bran	75 "
Wheat middlings	100 "
Soybean meal	70 "
Fish meal	60 "
Dehydrated alfalfa meal	75 "
Dried milk powder	20 "
Dried brewers' yeast	30 "
Salt	10 "
Fish oil	5 "
Rock phosphate	50 "
Limestone	30 "
Manganese sulphate	8 oz.

Scratch Grain Mixture

Whole wheat	1000lbs
Whole oats	400 "
Whole barley	600 "

Sample analysis of variance of egg-shell thickness data.

(Analysis for 20 p.p.m. level of antibiotic.)

Source	D. F.	S. S.	M. S.	F.	5% point
Treatments	3	18854.6	6284.9	149.1	2.61
Days	41	12611.5	307.6	7.3	1.41
Treatment X Days	123	6141.5	49.9	1.18	1.26
Error	1512	63740.9	42.15		
Total	1679	101348.5			

Analysis of variance for specific gravity data.

Data for 20. p.p.m. level of antibiotic.

Source	D. F.	S. S.	M. S.	F.	5% point
Treatments	3	0.0103	.00343	120.35	2.61
Days	41	.0116	.000283	9.93	1.41
Treatment X days	123	.00485	.0000394	1.38	
Error	1512	.0431	.0000285		
Total	1679	.0698			

Analysis of variance for blood calcium data

(20 p.p.m. level of antibiotic.)

Source	D. F.	S. S.	M. S.	F.	5% point
Treatments	3	1691.1	563.7	3.92	2.86
Error	36	5169.3	143.6		
Total	39	6860.4			

Analysis of variance for blood calcium data

(40 p.p.m. level of antibiotic.)

Source	D. F.	S. S.	M. S.	F.	5% point
Treatments	3	1418.5	472.8	3.79	2.86
Error	36	4482.1	124.5		
Total	39	5900.6			

Percent bone ash data for Experiment 3 (b)

	Treatments											
	Bacitracin			Penicillin			Terramycin			Basal		
	End of week			End of week			End of week			End of week		
	1	2	3	1	2	3	1	2	3	1	2	3
Rep.	28	30	33	33	36	29	33	34	29	39	32	34
I	31	30	29	37	32	28	32	32	30	36	29	32
	30	30	30	39	31	26	32	32	30	32	31	36
Total	89	90	92	109	99	83	97	98	89	107	92	102
Rep.	33	30	34	39	39	28	34	35	28	39	33	28
II	36	31	31	39	31	30	37	21	32	32	27	31
	39	31	30	40	35	30	37	28	30	35	30	32
Total	108	92	94	118	105	88	108	94	90	106	90	91
Tot. for treatment	565			602			576			588		

Analysis of variance for percent bone ash data. Experiment 3(b)

Source	D. F.	S. S.	M. S.	F.	5%
Treatment	3	42.1	14.0	1.36	3.71
Weekly periods	2	284.1	142.0	13.7	3.98
Treatment X weeks	6	138.8	23.1	2.24	3.09
Reps.	1	19.0	19.0	1.87	
Error	11	113.2	10.3		
Bet. birds within sample groups	48	304.7	6.3		
Total	71	901.9			

Weight gain data for Experiment 3 (b)

	Bacitracin			Penicillin			Terramycin			Basal		
	1	2	3	1	2	3	1	2	3	1	2	3
Rep.	37	51	64	32	34	59	34	47	62	23	49	62
I	24	45	71	26	50	65	27	48	70	33	63	50
	37	62	60	37	43	70	36	40	65	37	68	70
Total	98	158	195	95	127	194	97	135	197	93	180	182
Rep.	31	25	26	26	27	72	34	78	70	38	30	47
II	26	46	52	16	27	60	30	66	58	42	32	69
	31	40	62	32	50	52	38	51	65	33	46	62
Total	88	111	140	74	104	184	102	201	193	113	108	178
Tot. Rep. I & II	186	269	335	169	231	378	199	336	390	206	288	360
			790			778			925			854

Analysis of variance for weight gain data

Experiment 3(b)

Source	D. F.	S. S.	M. S.	F.	5% point
Treatments	3	662	221	1.01	3.71
Weeks	2	10300.3	5150.1	23.6	3.98
Treatments x weeks	6	709.2	118.2	.54	3.09
Replications	1	333.6	333.6	1.5	
Error	11	2400.1	218.2		
Bet. birds within sample groups	48	3588.7	747.6		
Total	71	17993.9			

Table of percent bone ash for Experiment 4.

	Basal						Penicillin						Terramycin					
	Low calcium			Low phos.			Low calcium			Low phos.			Low calcium			Low phos.		
	Weeks						Weeks						Weeks					
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Rep.	41	39	42	35	29	36	43	44	42	36	44	42	40	43	48	30	30	36
I	40	39	42	32	25	31	44	39	42	35	30	31	38	44	42	31	32	38
	40	39	43	30	31	35	40	41	42	30	29	27	40	43	43	29	36	36
Tot.	121	117	127	97	85	102	127	124	126	101	89	85	118	130	133	90	98	110
Rep.	38	40	38	37	31	35	42	41	43	33	37	32	39	40	42	32	32	32
II	38	38	41	35	36	37	36	41	41	34	26	39	39	41	42	31	30	31
	39	37	42	37	31	33	42	42	43	30	33	34	39	40	42	30	32	34
Tot.	115	115	121	109	98	105	120	124	127	97	96	105	117	121	126	93	94	97

Tot. Low ca 716 Tot. Low ph. 596 Tot. Low ca 748 Tot. Low ph. 573 Tot. Low ca. 745 Tot. Low ph. 582

Total Basal 1312 Total Penicillin 1321 Total Terramycin 1327

Analysis of variance for weight gains data. Experiment 4.

Source	D. F.	S. S.	M. S.	F.	5% point
A[1]	3	5669.5	1889.8	4.24	3.03
B[2]	1	8145.1	8145	18.29	4.28
C[3]	2	125268.5	62634.25	140.65	3.42
A[4]xB	3	2654.7	884.9	2.00	3.03
AxBxC	6	3440.8	573.5	1.28	2.53
AxC	6	6114	1019.1	2.29	2.53
BxC	2	3775.5	1887.8	4.23	3.42
Reps.	1	154.1	154.1	0.35	4.28
Error	23	10243.4	445.3		
Bet. birds within sample groups	96	20035.3	208.7		
Total	143	185500.9			

1 Antibiotic treatment

2 Low calcium and low phosphorous treatments

3 Weekly periods

4 etc. Interactions.

S. E. $= \sqrt{\frac{2 \times 445.3}{18}}$

$= 7.03$

$t_{.05}$ for 23 D.F. $= 2.07$

L S D $= 7.03 \times 2.07$

$= 14.55$

Least mean difference required for significance between low calcium treatments and between low phosphorous treatments

$= 14.55$

Weight gains for various treatments.

	Basal						Penicillin						Bacitracin						Terramycin					
	Low ca			Low ph.			Low ca			Low ph.			Low ca			Low ph.			Low ca			Low ph.		
	Week ending						Week ending						Week ending						Week ending					
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Rep.	26	55	45	28	30	59	29	62	80	22	40	67	22	72	144	22	24	109	11	14	155	20	55	109
I	13	63	92	15	34	66	38	98	151	29	45	81	30	52	110	10	55	71	25	64	134	15	60	82
	31	31	50	21	44	90	20	92	120	24	30	74	8	70	130	16	25	71	28	40	135	19	61	55
Tot.	70	149	187	64	108	215	87	252	351	75	115	222	60	194	384	48	104	251	64	118	424	54	176	246
Rep.	23	63	80	28	50	85	24	96	127	24	46	97	32	26	122	24	24	100	21	77	117	30	71	114
II	25	76	79	20	46	71	26	46	115	27	40	82	24	53	91	13	19	76	29	64	105	29	62	92
	30	59	82	32	61	81	17	69	158	30	38	101	24	15	81	16	30	49	34	81	103	39	34	92
Tot.	78	198	241	80	157	237	67	211	400	81	124	280	80	94	294	53	73	225	84	222	325	98	167	298
	148	347	428	144	265	452	154	463	751	156	239	502	140	288	678	101	177	476	148	340	749	152	343	544
			923			861			1368			897			1106			754			1237			1039
						1784						2265						1860						2276

Analysis of variance for percent bone ash data Experiment 4.

Source	D. F.	S. S.	M. S.	F.	5% point
A[1]	2	3.1	1.6	0.13	3.59
B[2]	1	1942.2	1942.2	160.5	4.45
C[3]	2	83.3	41.7	3.44	3.59
A X B[4]	2	46.5	23.3	1.92	3.59
A X C	4	89.1	22.3	1.84	2.96
B X C	2	22.8	11.4	.94	3.59
A X B X C	4	6.3	1.6	.13	2.96
Reps.	1				
Error	17	206	12.1		
Bet. birds within sample groups	72	322.7			
Total	107	2722.			

1 Antibiotic treatment

2 Low calcium and low phosphorous treatments

3 Weekly periods

4 etc. Interactions.

S. E.	$= \sqrt{\frac{2 \times 12.1}{18}}$
	$= 1.16$
$t_{.05}$ for 17 D.F.	$= 2.11$
L S D	$= 2.11 \times 1.16$
	$= 2.4$

Least main difference required for significance between low calcium treatments and between low phosphorous treatments under different main plot treatments. = 2.4