R9-1135-6m

TEXAS AGRICULTURAL EXPERIMENT STATION

A. B. CONNER, DIRECTOR

COLLEGE STATION, BRAZOS COUNTY, TEXAS

BULLETIN NO. 521 DECEMBER, 1935

DIVISION OF POULTRY HUSBANDRY
DIVISION OF CHEMISTRY

THE VITAMIN D REQUIREMENTS OF CHICKENS GROWN IN THE ABSENCE OF SUNLIGHT

AGRICULTURAL AND MECHANICAL COLLEGE OF TEXAS
T. O. WALTON, President

[Page Blank in Bulletin]

These experiments indicate that chickens fed a ration with the proper amounts of calcium and phosphorus need no additional vitamin D to make good growth for the first six weeks even in the absence of sunlight. The vitamin D requirement of the cockerel appears to be higher than that of the pullet. The vitamin D requirements of chickens vary with the particular criterion used in determining the effect of the vitamin D supplied. Maximum gain in weight required more vitamin D than any one of the other factors studied. The number of units of vitamin D required by growing chickens appears to depend upon the nature of the ration, especially the percentages of calcium and phosphorus contained in it. Chickens fed a ration containing 1.48 per cent calcium and 0.65 per cent phosphorus required 12.3 International units of vitamin D from cod liver oil per 100 grams of feed for maximum growth and best utilization of the feed, and 3.1 International units of vitamin D from cod liver oil per 100 grams of feed for the prevention of rickets and crooked breast bones and calcification of the bones at twelve weeks. Chickens fed a ration containing 0.96 per cent calcium and 0.66 per cent phosphorus required up to twelve weeks 50.2 International units of vitamin D from cod liver oil per 100 grams of feed for maximum growth, best utilization of the feed, and calcification of the bones, and 12.3 International units of vitamin D from cod liver oil per 100 grams of feed for the prevention of rickets and crooked breast bones. This high vitamin D requirement was probably due to the low calcium content of this ration. Chickens fed a ration containing 1.26 per cent calcium and 0.77 per cent phosphorus required up to twelve weeks 6.7 International units of vitamin D from cod liver oil per 100 grams of feed for maximum growth, prevention of crooked breast bones, best utilization of the feed, and calcification of the bones, and 3.4 International units of vitamin D per 100 grams of ration from cod liver oil for the prevention of rickets. The maximum level of vitamin D may not have been fed with this ration. Chickens fed a ration containing 1.36 per cent calcium and 0.78 per cent phosphorus required up to twelve weeks 6.7 International units of vitamin D from cod liver oil per 100 grams of feed for maximum growth and calcification of the bones, and 3.4 International units of vitamin D from cod liver oil per 100 grams of feed for the prevention of rickets and crooked breast bones and for best utilization of the feed. The maximum level of vitamin D may not have been fed with this ration.

CONTENTS

BULLETIN NO. 521 DECEMBER, 1935

THE VITAMIN D REQUIREMENTS OF CHICKENS GROWN IN THE ABSENCE OF SUNLIGHT

J. R. Couch, Associate Poultry Husbandman; G. S. Fraps, Chief Division of Chemistry; and R. M. Sherwood, Chief Division of Poultry Husbandry

Vitamin D or its equivalent is essential to the health of all animals. When insufficient amounts are furnished, a growing animal does not gain as rapidly as it should and the bones do not calcify properly, but remain soft and brittle. Vitamin D may be supplied as such or its equivalent may be furnished by sunlight or other light containing waves of the necessary length. Domestic animals, as a rule, do not suffer from deficiency of vitamin D since a sufficient amount is usually secured by an animal either from sunlight or from sun-cured feeds.

Some animals, such as children and chickens, are sometimes grown where they do not receive sufficient sunlight or where the active light is excluded by means of glass which absorbs it. Chickens grown in battery brooders do not receive sufficient vitamin D and require additions of vitamin D in order to make a good growth for good health. Vitamin D is usually furnished by means of cod liver oil or some other fish oil.

Chickens grown in the absence of sunlight and not fed a vitamin D supplement develop rickets and crooked breasts, their bones do not calcify normally, and they make poor growth. These troubles can be avoided if a sufficient quantity of vitamin D is included in the ration. This may be done by feeding fish oils which contain vitamin D, such as puffer fish oil, cod liver oil, salmon oil, and sardine oil, or by building brooder houses with sun porches so that the chickens may run in the sunlight which is the equivalent of vitamin D. They may be allowed to run on free range where they will get the equivalent of vitamin D from the sunlight. The equivalent of vitamin D may also be provided by irradiating the feed, by subjecting the chicks to ultraviolet light, or by feeding irradiated ergosterol.

The object of this study is to determine how much vitamin D will be required in rations fed chicks which do not have access to sunlight. Most poultrymen use one of the above-mentioned fish oils to furnish their chicks vitamin D. The fish oil manufacturers make recommendations as to how much of the oil should be fed to insure the chicks against rickets, crooked breast bones, and poor growth. The poultrymen try to feed a balanced ration, and the fish oil manufacturers' recommendations may be too high for such a ration. These fish oils are expensive and should not be fed in excess of the requirements of the chicken. When this study was begun, no data were available as to the quantity of vitamin D needed by growing chicks, but there has been considerable work reported since, as may be seen under the heading "Previous Work." For the solution of the problem it is necessary to know how much vitamin D the chick requires, how much of the antirachitic substance the chick can

get from the ration, and how much of a vitamin D supplement will be required to supply the vitamin D needed to meet the requirements of the growing chickens. Such knowledge may result in a great saving for the poultryman in feeding growing chicks, since the vitamin D supplement is one of the most expensive ingredients he has to buy. The question of whether or not a poultryman may grow his chicks in the absence of sunlight for a certain time and then put them out in the sun where they can get the equivalent of vitamin D will also be considered.

Previous Work

Hart, Steenbock, and Lepkovsky, and Halpin (13) in 1923 concluded that sunlight was equivalent to the antirachitic factor of food-stuffs in rearing chicks. They found that one-half hour daily exposure to direct sunlight was a better antirachitic equivalent than 5 per cent fresh green clover, calculated on the basis of the dry weight of the clover, fed in a synthetic ration. Bethke, Steenbock, and Nelson (4), working with rats in 1925 presented data which indicated the existence of a quantitative relation between the antirachitic vitamin and calcium in effecting the assimilation of the latter. Bethke and Kennard (1) in 1924-25 succeeded in rearing chicks indoors for over eight months by supplying them with cod liver oil or fresh egg yolk as sources of vitamin D. The pullets in the above study also produced a considerable number of eggs. Karelitz and Sgohl (16) in 1927 were able to cause a rapid healing of rickets in rats by the addition of phosphate to a rickets-producing diet with vitamin and light factors unchanged. Bethke, Kennard, Kick, and Zinzalian (1929) (2) concluded that the optimum or near optimum ratio of calcium to phosphorus for the growing chick lies between 3:1 and 4:1, and said that the requirements for the antirachitic factor were at a minimum with this relationship. Wilgus (1931) (30) concluded that when an optimum supply of the antirachitic factor is provided, the calcium requirements of the growing chick approach the minimum level of .66 per cent; that the calcium-phosphorus ratio may vary between 1:1 and 2.2:1 with normal results; and that a ratio of 2.5:1 appeared to be on the border line while a ratio of 3.3:1 was disastrous. Sherwood (1932) (27) found that for normal growth growing chicks required from 1.5 to 3.0 per cent of lime (equivalent to 1.07-2.14 per cent of calcium), and from 1.5 to 2.0 per cent of phosphoric acid (equivalent to .65-.87 per cent of phosphorus). Sherman and Stiebeling (29) in 1930 concluded that either vitamin D, calcium, or phosphorus might prove to be the limiting factor in the development of bone in rats. Brown and Shohl (6) in 1930 concluded that the amount of calcium and phosphorus in the diet determined the retention of these elements in the body of the rat, and that vitamin D controlled their intermediary metabolism. In 1931 Holmes and Pigott (15) reported that cod liver oil was very effective in promoting calcium utilization in the chicken on a ration with a low calcium-phosphorus ratio but was less effective with a high calcium-phosphorus ratio. Bethke, Record, and Kennard (1933) (3)

concluded that the chick required for normal calcification a minimum of approximately 18.9 International units of vitamin D from cod liver oil per 100 grams of the ration. They used two rations in their work. The first was composed of 52 parts ground yellow corn, 20 parts ground wheat, 5 parts wheat bran, 20 parts dried buttermilk, 2 parts calcium carbonate, and 1 part sodium chloride; this analyzed 1.02 per cent calcium and 0.45 per cent phosphorus. The second was made up of 46 parts ground yellow corn, 20 parts ground wheat, 5 parts wheat bran, 20 parts soybean oilmeal, 5 parts dried buttermilk, 3 parts steamed bone meal, and 1 part sodium chloride; this one analyzed 1.12-1.36 per cent calcium and 0.88-0.98 per cent phosphorus. Murphy, Hunter, and Knandel (1934) (22) using a ration composed of 43.5 pounds ground yellow corn, 10 pounds wheat bran, 10 pounds standard wheat middlings, 5 pounds alfalfa leaf meal, 10 pounds 55 per cent meat and bone scraps, 10 pounds dried milk, 10 pounds ground heavy oats, 1 pound ground limestone, and .5 pound salt, which ration analyzed 1.47 per cent calcium and 0.92 per cent phosphorus, concluded that Single Comb White Leghorn chicks deprived of sunshine required a minimum protective level of 17 International units of vitamin D from cod liver oil per 100 grams of ration from hatching time to 24 weeks of age. Carver, Robertson, Brazie, Johnson, and St. John (1934), (7) using a ration composed of 40 pounds ground yellow corn, 10 pounds ground wheat, 10 pounds ground oats, 15 pounds white wheat bran, 5 pounds dehydrated alfalfa leaf meal, 10 pounds meat scrap, 8 pounds skim milk powder, 1 pound ground oyster shell, and 1 pound salt, which ration analyzed 2.5 per cent calcium and 0.90 per cent phosphorus, concluded that growing pullets in confinement without sunlight required a minimum of approximately 17 International units of vitamin D from cod liver oil per 100 grams ration for satisfactory calcification and growth. Russell, Taylor, and Wilcox (1934), (24) using a ration composed of 47 per cent ground yellow corn, 20 per cent wheat middlings, 15 per cent wheat bran, 9 per cent meat scrap, 5 per cent dried skim milk, 3 per cent oyster shell, and 1 per cent sodium chloride, concluded that normal bone ash and body weight were obtained in chickens at 8 weeks of age when 0.25 per cent cod liver oil or 14.9 International units of vitamin D per 100 grams of ration was fed. Bethke, Record, and Kennard (3), and Steenbook, Kleitzen, and Halpin (26) have also determined that the chicken is not able to utilize irradiated ergosterol or irradiated yeast as efficiently as the vitamin D of fish oils. Russell, Taylor, and Wilcox (24) found the same to be true of irradiated ergosterol.

Method of Procedure

In 1932-33 two rations which differed widely in their ingredients so as to represent different feeds were each fed to duplicate groups of chicks. Ration 1 analyzed 1.48 per cent calcium and .65 per cent phosphorus with a calcium-phosphorus ratio of 1:.44. Ration 2 analyzed .96 per cent calcium and .66 per cent phosphorus with a calcium-phosphorus ratio of 1:.69. In 1933-34 a ration with the same percentage ingredients as ration 2 was fed in duplicate. However, this ration is

designated as ration 3 because it had a different content of calcium and phosphorus. This difference is due to the use of a different lot of meat and bone scrap which was much higher in ash and consequently much higher in lime and phosphoric acid than that used in ration 2. Ration 3 analyzed 1.26 per cent calcium and .77 per cent phosphorus with a calcium-phosphorus ratio of 1:.61. Following this experiment in the same year, a fourth ration was fed to duplicate groups in one experiment. Ration 4 analyzed 1.36 per cent calcium and .78 per cent phosphorus with a calcium-phosphorus ratio of 1:.57. The calcium and phosphorus content of rations 1, 2, 3, and 4 are in agreement with the requirements found in the work of Sherwood (1932) (27), although the calcium and phosphorus content of these rations and the ratio of calcium to phosphorus is somewhat higher than that reported as most favorable by Bethke, Kennard, Kick, and Zinzalian (1929) (2); by Hart, Scott, Kline, and Halpin (1923) (14); and by Wilgus (1931) (30).

Day-old chicks were divided into groups of about 25 each and fed only upon the selected ration supplemented with various amounts of fortified cod liver oil to supply the vitamin D. Vitamin D was determined in the cod liver oil and in the rations with rats as the test animals. Average gains in weight at six, eight, and twelve weeks; percentage of chicks with rickets and crooked breast bones; grams of feed required to produce one gram of gain; and the analysis of the bones for percentage of bone ash were taken into consideration in deciding upon the effect of the vitamin D supplied.

The chicks used in these experiments were Single Comb White Leghorns of the same breeding and were from the stock maintained at the Poultry Division of the Texas Agricultural Experiment Station. The chicks were taken from the incubator on the hatching date and weighed, and only those weighing from 33 to 45 grams were used for experimental purposes. Lots were made up by distributing the chicks at random among twelve lots. They were kept in an electrically-heated battery brooder for the first two weeks. They were subsequently moved to larger batteries as they required more space. These brooders were situated in a brooder house that was built for experimental purposes. The windows are glazed with ordinary window glass and are covered with white duck on the inside to largely exclude outside light. The brooder and batteries were placed in such a way that each lot of chicks had as nearly uniform conditions as it was possible to maintain. The temperature was maintained as nearly as possible at 75° Fahrenheit, but when the outside temperature was above 75°, it was not possible to keep the inside of the building at this temperature.

The chicks were weighed at the beginning of the experiment and at intervals of two weeks for the twelve weeks of the experiment. They were weighed early in the forenoon after the feed had been withheld since six o'clock the preceding afternoon. The chicks had an opportunity to feed approximately eleven hours per day. Feed was weighed to the chicks daily, and they were fed all they would consume. Tap water was kept before them at all times.

The percentage of ash was run on the bones of representative chicks from each group at twelve weeks to determine the degree of calcification in various groups that were receiving different amounts of vitamin D. The bones of the chicks in a particular group were considered as being completely calcified when increasing amounts of vitamin D from cod liver oil failed to produce an increase in the percentage of bone ash. This was used in estimating the vitamin D requirements of the chicks. The analysis of the bones was made in the Division of Chemistry of the Texas Agricultural Experiment Station.

VITAMIN D IN FEED AND SUPPLEMENT

The units of vitamin D were estimated in the cod liver oils used and in a thoroughly mixed sample of each of the rations. The albino rats used for this determination were from the stock colony of the Texas Agricultural Experiment Station rat laboratory and were raised on the following ration: corn meal 1200 grams, cottonseed meal 300 grams, powdered whole milk 600 grams, alfalfa leaf meal 40 grams, precipitated calcium carbonate 10 grams, ferric citrate 1 gram, and copper sulphate 0.5 gram, with tap water ad libitum; they were fed spinach once each week.

The method for the determination of vitamin D was based on the work of McCollum, Simmonds, Shipley, and Park (17), Steenbock and Black (25), Bills, Honeywell, Wirick, and Nussmeier (5), and Munch (21). The young rats weaned at 22-26 days old, and weighing from 38 to 48 grams, were placed in a caged-in screened stack from which outside light was excluded by covering the screens with black cloth. The rats received Steenbock's (25) rickets-producing diet 2965, which was composed of ground yellow corn 76 parts, wheat gluten 20 parts, precipitated calcium carbonate 3 parts, and sodium chloride 1 part, with distilled water to drink. At the end of the depletion period of 21 days, a number of rats were killed (see negative controls, Table 1), and the tibiae examined by the method of McCollum and Simmonds, and Shipley and Park (17), which consisted of immersing the bones in a 2 per cent solution of silver nitrate, placing under a strong light, dipping in a 5 per cent solution of sodium thiosulfate, and examining under a binocular microscope for deposits of black silver on the bone. The other rats were arranged in groups of six, and the substance to be tested was fed in addition to diet 2965 each day except Sunday until the rat had received eleven feedings. The cod liver oil fed was diluted with cottonseed oil so that 0.1 cc was fed each day. The rats were weighed when placed on experiment, every seven days during the course of the experiment, and at the completion of the test. Diet 2965 was weighed to the individual rats at weekly intervals and the amount consumed was recorded. At the completion of the test, the rats were killed and the tibiae examined as described above.

The scale used by Bills, Honeywell, Wirick, and Nussmeier (5) was used in this study. The symbols +, ++, +++, and ++++ express different degrees of healing. A + bone is one that shows some calcifica-

tion but is a rachitic bone; a ++ bone is one that has a narrow but continuous line of calcium; a +++ bone is one which has a heavier line than the ++ bone; and a ++++ bone is one that is calcified normally. The minus sign (—) was used to designate a bone that was distinctly rachitic and did not show any calcification. When tests showed that the amount of cod liver oil fed produced an average ++ bone or one with a narrow and continuous line of calcium deposits in the metaphyses of the distal ends of the radii and ulnae of standard rachitic rats the amount of vitamin D was equal to 0.4 of an International unit.

The No. 1 fortified cod liver oil used in these experiments was estimated to contain 200 International rat units of vitamin D per gram. Nine groups of six rats each were used in this test (Table 1) and this estimation was arrived at from the group of rats fed .002 grams of oil, which was estimated to contain 0.4 of an International unit of vitamin D.

Table 1. Results of vitamin D determination in cod liver oils and in rations

Amount fed	Degree of healing of each rat					
Negative controls	—,	+++,	+,	+,	+,	—,
" "	—,	—,	—,	—,	—,	.....
" "	—,	++,	—,	—,	—,	—,
.07 grams cod liver oil No. 1	++++,	++++,	++++,	++++,	++++,	++++,
.04 " " " " " 1	++++,	++++,	++++,	++++,	++,	++++,
.02 " " " " " 1	++++,	++++,	++++,	++++,	++,	++++,
.01 " " " " " 1	+++,	++,	++++,	++++,	++++,	+++,
.005 " " " " " 1	+++,	++++,	++++,	+++,	+++,	++,
.004 " " " " " 1	++,	+++,	+++,	++,	++,	++,
.003 " " " " " 1	++,	+,	++,	+++,	++,	+++,
.002 " " " " " 1	++,	++,	++,	++,	+,	+++,
.0016 " " " " " 1	—,	++,	+,	+,	+,	+,
.001 " " " " " 1	—,	—,	—,	—,	—,	+++,
.02 " " " " " 2	++,	++,	++,	+++,	++,	++,
.015 " " " " " 2	++,	++,	++,	+++,	++,	++,
.01 " " " " " 2	+,	+,	+,	+,	+,	—,
.005 " " " " " 2	+++,	+,	+,	+,	+,	++,
.004 " " " " " 2	+,	+,	—,	++,	++,	+,
Ration 1 fed ad lib						
8.4 grams per day consumed	++++,	++++,	++++,	++++,	++++,	++++,
5 grams ration 1	++++,	++,	++++,	++,	+++,	.....
3 grams ration 1	++,	++,	++,	++,	+++,	++,
2 grams ration 1	+,	+,	+,	+,	.....	.....
Ration 2 fed ad lib						
11.6 grams per day consumed	++++,	++++,	++++,	++++,	++++,	++++,
5 grams ration 2	++,	+++,	+++,	+++,	+++,	++,
Ration 3 fed ad lib						
9.9 grams per day consumed	++++,	++++,	++++,	++++,	++++,	++++,
5 grams ration 3	++,	+,	+,	++,	+,	+,
6 grams ration 3	++,	++,	++++,	+++,	++++,	—,
Ration 4 fed ad lib						
12.2 grams per day consumed	++++,	++,	++++,	++++,	++++,	++,
5 grams ration 4	—,	+,	+,	+,	+,	+++,

The No. 2 cod liver oil used in these experiments was estimated to contain 26.7 International units of vitamin D per gram. Five groups of six rats each were used in this test (Table 1) and this estimate was arrived at from the group of rats fed .015 grams of oil, which was estimated to contain .4 of an International unit of vitamin D.

The authors are unable to say to what extent the calcification of the bones in rachitic rats produced by rations 1, 2, 3, and 4 is due to vitamin D and to what extent it is due to favorable amounts of calcium and

Table 2. Percentage of ingredients in rations

	Ration numbers			
	1	2	3	4
Ground kafir and milo	.....	.....	.....	55
Ground milo	53	.....	.....	.....
Dehydrated alfalfa leaf meal	5	5	5	5
Dried buttermilk	18	.....	.....	6
Ground oyster shell	2	1	1	1
Bone meal	1	.....	.....	.....
Wheat gray shorts	20	20	20	20
Salt	1	1	1	1
Yellow corn meal	.....	55	55	.....
Dried skimmed milk	.....	6	6	.....
Meat and bone scrap No. 1	.....	6	.....	.....
Meat and bone scrap No. 2	.....	.....	6	6
Cottonseed meal	.....	6	6	6

phosphorus. Ration 1, Table 2, was estimated to contain the equivalent of one International unit of vitamin D in 7.5 grams of ration. One group of four rats, one group of five rats, and two groups of six rats each were used in testing ration 1 for vitamin D (Table 1). Ration 2, Table 2, was estimated to contain the equivalent of one International unit of vitamin D per 10 grams of ration. Two groups of six rats each were used in making this determination. Ration 3, Table 2, was estimated to contain the equivalent of one International unit of vitamin D per 13.8 grams of ration. Three groups of six rats each were used in making this determination. Ration 4, Table 2, was estimated to contain the equivalent of one International unit of vitamin D per 15 grams of ration. Two groups of six rats each were used in making this determination.

VITAMIN D REQUIREMENTS OF CHICKENS FED RATION 1

Ration 1 (Table 2) unsupplemented and with varying amounts of No. 1 fortified cod liver oil (Table 1)—1/64 per cent, 1/32 per cent, 2/32 per cent, 3/32 per cent, and 4/32 per cent—was fed to duplicate groups of

Table 3. Percentage composition of rations

	Ration numbers			
	1	2	3	4
Protein	19.01	18.35	18.21	20.02
Fat	4.06	4.22	4.42	1.40
Crude fiber	3.77	3.91	3.93	3.62
Nitrogen free extract	57.21	59.03	59.87	57.52
Water	9.37	9.01	7.10	10.49
Ash	0.58	5.48	6.47	6.95
Lime (Ca 0)	2.07	1.35	1.77	1.91
Total phosphoric acid (P_2O_5)	1.49	1.51	1.77	1.79
Calcium	1.48	0.96	1.26	1.36
Phosphorus	0.65	0.66	0.77	0.78
Lime: Phosphoric acid ratio	1:.72	1:1.12	1:1.00	1:.94
Calcium: Phosphorus ratio	1:.44	1:.69	1:.61	1:.57

chicks in two experiments. These amounts of No. 1 cod liver oil contained 3.1, 6.2, 12.3, 19.0, and 25.1 International units of vitamin D respectively per 100 grams of mixed feed (figured from the rat data of Table 1). Ration 1 was estimated to contain 13.3 International units of vitamin D equivalent per 100 grams as figured from the rat data of Table 1. The chicks in the group on the unsupplemented ration are designated as the no-vitamin D group, and the other groups are designated according to the units of vitamin D from the fortified cod liver oil that they received per 100 grams of ration.

Gains in Weight at Six, Eight, and Twelve Weeks

It may be noted from tables 4, 6, and 8 that the differences between the various groups in the gains of the chicks at six weeks in experiments 1 and 2 are small and insignificant in most cases. There was no signifi-

Table 4. Relation of vitamin D in feed to gain in weight of chickens fed ration 1, experiment 1

International units of vitamin D from fortified cod liver oil per 100 grams of ration	Mean gain in grams					
	Cockerels			Pullets		
	Six weeks	Eight weeks	Twelve weeks	Six weeks	Eight weeks	Twelve weeks
0	307.2± 6.28	470.3± 9.02	756.7±14.80	273.6± 5.17	401.4± 7.33	588.0±15.57
3.1	298.1± 6.71	515.1±10.60	925.4±19.14	265.3± 5.10	439.2± 7.31	756.7±13.15
6.2	321.9± 7.32	515.9±10.61	938.9±14.37	273.9± 5.32	431.9± 7.75	741.5±12.76
12.3	335.2± 4.50	549.4± 7.35	1003.3±10.31	289.6± 6.18	455.2± 8.87	783.4±11.34
19.0	315.6± 6.78	532.6±10.13	969.4±14.76	278.8± 5.72	448.6± 8.86	776.7±12.78
25.1	324.3± 6.62	522.3± 9.52	968.3±14.00	287.4± 7.09	452.1±11.11	778.3±13.31

cant difference between the gains of the no-vitamin D groups and the 25.1-vitamin D groups of either cockerels or pullets in experiment 1 (Table 4). The mean differences in the gains of the cockerels and pullets of experiment 2 (Table 6) between the no-vitamin D groups and the 3.1-vitamin D groups are three times their probable errors. However,

Table 5. Relation of vitamin D to rickets and to other criteria on chickens fed ration 1, experiment 1

International units of vitamin D from fortified cod liver oil per 100 grams of ration	Percentage of leg deformities at twelve weeks		Percentage of the chicks that had crooked breast bones at 12 weeks	Number of chicks that died during experiment	Grams of feed required to produce one gram of gain	Mean percentage of bone ash
	rickets	slipped tendons				
0	50.0	0	40.0	0	4.26	40.71
3.1	2.1	0	0	3	4.05	46.16
6.2	0	0	0	2	3.89	45.33
12.3	0	8.2	2.0	1	3.76	46.34
19.0	0	2.1	0	2	3.84	46.95
25.1	0	0	0	4	3.92	46.58

a more significant fact about these results is that though the differences between groups are small, the gains of the cockerels and pullets at six weeks in the no-vitamin D groups of experiments 1 and 2 (Tables 4 and 6) are in most cases smaller than the gains of the groups receiving vitamin D from cod liver oil. Therefore, the chicks in the no-vitamin D

Table 6. Relation of vitamin D in feed to gain in weight of chickens fed ration 1, experiment 2

International units of vitamin D from fortified cod liver oil per 100 grams of ration	Mean gain in grams					
	Cockerels			Pullets		
	Six weeks	Eight weeks	Twelve weeks	Six weeks	Eight weeks	Twelve weeks
0	284.2± 5.62	435.6± 6.76	691.8±12.01	248.2± 7.72	367.2± 9.78	548.1±20.11
3.1	311.0± 3.76	483.1± 8.95	856.4±13.47	278.8± 6.39	421.7±10.27	711.0±11.18
6.2	303.2± 5.87	486.9± 7.48	891.0±11.04	262.0± 5.09	397.7± 6.09	664.9±18.57
12.3	323.8± 6.89	530.9± 9.61	941.7±21.14	272.3± 6.15	416.1± 8.44	714.0±13.47
19.0	306.5± 6.68	498.1± 8.64	884.2±16.51	282.3± 5.55	429.1± 6.61	709.4±11.49
25.1	316.5± 6.06	504.1± 7.93	925.5±14.28	286.8± 7.44	418.6±10.49	733.9±15.19

groups were probably not receiving enough vitamin D from the basal ration for the best growth up to six weeks, although there were no visible signs of rickets in these groups at six weeks, and the chicks appeared to be in good physical condition. The vitamin D requirements of growing chicks fed ration 1 at six weeks is estimated to be between 0 and 3.1 International units of vitamin D from cod liver oil per 100 grams of ration. The chicks in the no-vitamin D groups might not have needed any additional vitamin D up to six weeks if at this time they had been given access to direct sunlight or if a vitamin D supplement had been included in the ration after this time.

At eight weeks there is a significant difference in the gains of the chicks fed ration 1 between the no-vitamin D groups and the 3.1-vitamin D groups of experiments 1 and 2 (Tables 4, 6, and 8). At eight weeks there is no significant difference in the gains of the chicks between the 3.1-vitamin D groups and any of the groups receiving higher levels of vitamin D. The chicks in the no-vitamin D groups showed slight symptoms of rickets at eight weeks, but there was no evidence of this defect in any of the groups receiving various amounts of vitamin D from cod liver oil. The chicks in the 3.1-vitamin D groups were receiving sufficient vitamin D for proper growth and prevention of rickets at eight weeks. These chicks were receiving 3.1 units of vitamin D from cod liver oil per 100 grams of ration at eight weeks.

At twelve weeks there is a significant difference in the gains of the chicks of experiments 1 and 2 between the no-vitamin D groups and the 3.1-vitamin D groups (Tables 4, 6, and 8). There is a significant difference between the gains of the cockerels in the 3.1-vitamin D group and the gains of those in the 12.3-vitamin D group of experiment 1 (Table 4) at twelve weeks. There is no significant difference between the cockerels

in the 3.1-vitamin D group and those of any other group receiving higher levels of vitamin, with the exception of the cockerels in the 12.3-vitamin D group. There is no significant difference between the gains of the pullets in the 3.1-vitamin D group and the gains of those receiving higher levels of vitamin D in experiment 1 (Table 4), although the mean gains tend to increase up to the group receiving 12.3 units of vitamin D per 100 grams of ration. There is a significant difference in the gains of the cockerels in experiment 2 at twelve weeks between the 3.1-vitamin D group and the 12.3-vitamin D group and between the 3.1-vitamin D group and the 25.1-vitamin D group (Table 6). There is no significant difference in the gains of the pullets in experiment 2 at twelve weeks between the 3.1-vitamin D groups and other groups receiving higher levels of vitamin D (Table 6); but the gains of the pullets in the 12.3, 19.0, and 25.1-vitamin D groups are larger than that of the 3.1-vitamin D group.

From the data presented in Tables 4 and 6 one could conclude that growing chickens fed ration 1 required for proper growth up to twelve weeks a minimum of 3.1 International units of vitamin D from fortified cod liver oil per 100 grams of feed, but it may be noted from Table 8 that the mean gain of cockerels and pullets at twelve weeks in the 12.3-vitamin D groups was consistently larger than the mean gain of the cockerels and pullets in the 3.1-vitamin D groups. In view of the fact that each of these experiments was run in duplicate and that they both check very closely on this point, it would be safer to conclude that growing chickens fed ration 1 required for the best growth 12.3 International units of vitamin D from fortified cod liver oil per 100 grams of feed. The value of the added gains of the 12.3-vitamin groups would usually much more than pay for the cod liver oil needed to supply the additional vitamin D.

There is a tendency for the cockerels in experiments 1 and 2 (Tables 4 and 6) to respond better than the pullets to additions of vitamin D above that of the 3.1-vitamin D group. This might indicate that the requirement of the cockerels for vitamin D is higher than the requirement of the pullets. Such an indication is quite plausible since the sexual development of the cockerel takes place so much earlier than that of the pullet.

Leg Deformities and Crooked Breast Bones at Twelve Weeks

With but one exception the chicks in the no-vitamin D groups were the only ones that showed visible signs of rickets at twelve weeks (Tables 5 and 7). The average percentage of rickets of the no-vitamin D groups is 50.0 in experiment 1 (Table 5) and 30.6 in experiment 2 (Table 7).

A large percentage of the chicks in the no-vitamin D groups had crooked breast bones at twelve weeks (Tables 5 and 7). The average percentage of chicks with crooked breast bones in the no-vitamin D groups of experiment 1 (Table 5) was 40.0, and of experiment 2 (Table 7) was 61.2. Smaller percentages of chicks with this defect were noted in some of the

Table 7. Relation of vitamin D to rickets and to other criteria on chickens fed ration 1, experiment 2

International units of vitamin D from fortified cod liver oil per 100 grams of ration	Percentage of leg deformities at twelve weeks		Percentage of the chicks that had crooked breast bones at 12 weeks	Number of chicks that died during experiment	Grams of feed required to produce one gram of gain	Mean percentage of bone ash
	rickets	slipped tendons				
0	30.6	0	61.2	3	4.30	41.79
3.1	0	4.4	0	6	3.99	45.92
6.2	0	2.0	0	1	3.94	46.10
12.3	0	0	2.1	4	3.88	*
19.0	0	5.8	3.9	0	3.86	*
25.1	0	2.0	2.0	3	3.87	*

groups receiving higher levels of vitamin D than in the 3.1-vitamin D group (Tables 5 and 7), but these percentages were possibly due to other causes than lack of vitamin D. For the prevention of rickets and crooked breast bones at twelve weeks, the chick requires a minimum of approximately 3.1 units of vitamin D from fortified cod liver oil per 100 grams of ration 1.

Mortality, Grams of Feed Required to Produce One Gram of Gain, and Percentage of Bone Ash at Twelve Weeks

The number of chicks that died during the experimental period was small and apparently was not related to the vitamin D intake (Tables 5 and 7). There was a total of 612 chicks in the two experiments at the beginning, and only 29 died during the twelve-week period.

There is a significant difference in the grams of feed required to produce 1 gram of gain when the no-vitamin D groups and the 3.1-vitamin D groups of experiments 1 and 2 (Tables 5 and 7) are compared, and there is an appreciable difference in the grams of feed required to produce one gram of gain when the 3.1-vitamin D groups and the other groups receiving higher levels of vitamin D are compared. For proper utilization of the feed, growing chicks fed ration 1 (Table 2) required between 6.2 and 12.3 International units of vitamin D from fortified cod liver oil per 100 grams of ration.

The chicks in the 3.1-vitamin D group were receiving adequate vitamin D for complete calcification of the bones at twelve weeks, since the addition of amounts of vitamin D up to 25.1 units from fortified cod liver oil did not increase the percentage of bone ash above that of the 3.1-vitamin D group (Tables 5 and 7). The chicks in the no-vitamin D group were not receiving sufficient vitamin D for complete calcification of the bones at twelve weeks, since the percentage of bone ash of the no-vitamin D group in experiment 1 (Table 5) is 40.71 and of the 3.1-vitamin D group is 46.16. The percentage of bone ash of the no-vitamin D group in experi-

*Bone ash determinations were not run for these groups since there were no significant difference in the percentage of bone ash between the 3.1-vitamin D group and the groups receiving higher levels of vitamin D in experiment 1.

Table 8. Relation of vitamin D to gain in weight of chickens fed ration 1, experiments 1 and 2 combined

International units of vitamin D from fortified cod liver oil per 100 grams of ration	Mean gain in grams					
	Cockerels			Pullets		
	Six weeks	Eight weeks	Twelve weeks	Six weeks	Eight weeks	Twelve weeks
0.0	295.7	453.0	724.3	260.9	348.3	568.1
3.1	304.6	499.1	890.9	272.1	430.5	733.9
6.2	312.6	504.1	915.0	268.0	414.8	703.2
12.3	329.5	540.2	972.5	281.0	435.7	748.7
19.0	311.1	515.4	926.8	280.6	438.9	743.1
25.1	320.4	513.2	946.9	287.1	435.4	756.1

ment 2 (Table 7) is 41.79 and of the 3.1-vitamin D group is 45.92. An increase of 5.45 per cent in the percentage of bone ash in experiment 1 and an increase of 4.13 per cent in the percentage of bone ash in experiment 2 were made by the addition of 3.1 units of vitamin D per 100 grams of ration 1, but no appreciable increase was noted in the percentage of bone ash with additions of vitamin D above this. Thus it is seen that for complete calcification of the bones as determined by percentage of bone ash at twelve weeks, growing chicks fed ration 1 required a minimum of approximately 3.1 International units of vitamin D from fortified cod liver oil per 100 grams of ration.

VITAMIN D REQUIREMENTS OF CHICKENS FED RATION 2

Ration 2 (Table 2) unsupplemented and with varying amounts of No. 1 fortified cod liver oil (Table 1)—1/64 per cent, 1/32 per cent, 1/16 per cent, 1/8 per cent and 1/4 per cent—was fed to duplicate groups of chicks in two experiments. The above-mentioned amounts of fortified cod liver oil contained 3.1, 6.2, 12.3, 25.1, and 50.2 International units of vitamin D respectively per 100 grams of mixed ration as figured from the rat data of Table 1. Ration 2 was estimated to contain 10 International units of vitamin D equivalent per 100 grams of feed. The chicks on the unsupplemented ration are designated as the no-vitamin D group and the other groups are designated according to the units of vitamin D that they received from the fortified cod liver oil per 100 grams of ration.

Gains in Weight at Six, Eight, and Twelve Weeks

There was a decided increase in the gains of the chicks in the 12.3-vitamin D groups of experiment 3 (Table 9) at six weeks over those of groups receiving lower amounts of vitamin D, and there was a decided increase in the gains at six weeks of the chicks of the 6.2-vitamin D groups, experiment 4 (Table 11), over those in the groups receiving lower amounts of vitamin D. There was very little difference between the

12.3-vitamin D groups of experiment 3 and the groups receiving higher levels of vitamin D in the gains of the chicks at six weeks.

Table 9. Relation of vitamin D in feed to gain in weight of chickens fed ration 2, experiment 3

International units of vitamin D from fortified cod liver oil per 100 grams of ration	Mean gain in grams					
	Cockerels			Pullets		
	Six weeks	Eight weeks	Twelve weeks	Six weeks	Eight weeks	Twelve weeks
0	276.5± 7.73	396.4±11.59	586.2±18.10	226.9± 8.28	319.6±11.37	449.3±16.44
3.1	262.1±10.45	368.8±14.37	585.6±29.23	209.0± 6.00	289.2± 9.53	468.5±19.50
6.2	264.7± 7.09	418.8±11.96	669.1±28.56	218.4± 6.69	334.4± 9.91	546.3±19.23
12.3	293.1±10.18	454.0±19.76	795.2±39.28	262.1± 7.19	416.7± 9.11	721.3±16.81
25.1	296.4± 9.32	474.2±13.96	839.2±28.14	257.6± 8.16	386.6±14.22	732.2±14.08
50.2	318.0±10.16	502.5±24.84	944.1±20.61	281.9± 7.22	406.7±11.63	664.3±25.94

It may be noted from Tables 9, 11, and 13 that there is a tendency for the gains of the chicks to continue to increase with increasing amounts of vitamin D up to the highest amount fed. The differences between the groups with each increase of vitamin D are small, but they are so large that the mean differences between the no-vitamin D groups and the 50.2-vitamin D groups are significant. Therefore, the chicks in the no-vitamin D groups were not receiving sufficient vitamin D for proper growth at six weeks.

Table 10. Relation of vitamin D to rickets and to other criteria on chickens fed ration 2, experiment 3

International units of vitamin D from fortified cod liver oil per 100 grams of ration	Percentage of leg deformities at twelve weeks		Percentage of the chicks that had crooked breast bones at 12 weeks	Number of chicks that died during experiment	Grams of feed required to produce one gram of gain	Mean percentage of bone ash
	rickets	slipped tendons				
0	58.8	0	50.0	10	4.91	38.43
3.1	46.0	0	40.5	8	4.43	38.18
6.2	16.7	0	4.8	4	4.06	43.79
12.3	0	0	0	9	3.74	43.07
25.1	0	0	0	6	3.58	45.23
50.2	0	2.6	0	7	3.86	47.24

From these data, it is concluded that for growth up to six weeks growing chicks fed ration 2 required between 6.2 and 12.3 International units of vitamin D from fortified cod liver oil per 100 grams of ration.

The chicks in the no-vitamin D groups might not have needed any additional vitamin D beyond that which they were receiving at six weeks if after this time they had been given access to direct sunlight or if a vitamin D supplement had been included in the ration at six weeks.

There was very little difference in the gains of the chicks of experiments 3 and 4 (Tables 9, 11, and 13) at eight weeks between the no-vitamin D

Table 11. Relation of vitamin D in feed to gain in weight of chickens fed ration 2, experiment 4

International units of vitamin D from fortified cod liver oil per 100 grams of ration	Mean gain in grams					
	Cockerels			Pullets		
	Six weeks	Eight weeks	Twelve weeks	Six weeks	Eight weeks	Twelve weeks
0	253.4± 6.09	359.7± 9.05	529.8±17.09	215.6± 6.47	279.1± 9.79	380.8±13.52
3.1	255.4± 7.82	341.9±10.47	530.7±25.11	213.2± 4.95	285.7± 8.06	421.1±20.04
6.2	277.2± 8.96	377.0±27.24	669.0±51.15	238.9±13.43	334.1±14.58	492.4±29.27
12.3	274.3± 9.13	421.5±25.65	755.8±29.25	227.8± 7.71	335.7±13.92	560.5±32.61
25.1	278.9±12.46	445.1±19.54	795.1±35.54	237.6± 7.67	348.2±13.94	641.5±22.03
50.2	287.4± 7.65	447.3±11.52	861.7±14.58	235.9± 5.53	353.9± 8.56	656.3±14.52

group, the 3.1-vitamin D groups, and the 6.2-vitamin D groups, with one exception, which was the pullets in the 6.2-vitamin D groups of experiment 4 (Table 11). The gains of the cockerels increased with increasing amounts of vitamin D up to the 50.2-vitamin D groups in both experiments 3 and 4 (Tables 9 and 11). The pullet gains failed to increase with increasing amounts of vitamin D above that of the 12.3-vitamin D groups in experiment 3 and did not increase appreciably above that of the 6.2-vitamin D groups in experiment 4. There was a decided increase at eight weeks in the gains of the chicks of experiments 3 and 4 in the 12.3-vitamin D groups over the gains of those receiving lower levels of vitamin D.

There was very little difference at twelve weeks between the gains of the chicks of experiments 3 and 4 (Tables 9, 11, and 13) in the no-vitamin D groups and those of the 3.1-vitamin D groups. There was a decided increase in the gains of the chicks in the 6.2-vitamin D groups over those of the first-mentioned groups showing the effect of additional vitamin D from cod liver oil. There is a significant difference in the gains of the cockerels at twelve weeks between the no-vitamin D groups and the 50.2-vitamin D groups of both experiments 3 and 4 (Tables 9 and 11). From these data it cannot be said that the optimum level of vitamin D for the cockerels was fed, because there was an increase in the gains of the various groups of cockerels with additional amounts of vitamin D up to the 50.2-vitamin D group, which received the highest amount of vitamin D of any of the groups. This was not true with the pullets. Here again there is an indication that the cockerel's requirement for vitamin D is higher than that of the pullet. There is a significant difference in the gains of the pullets at twelve weeks between the no-vitamin D groups and the 12.3-vitamin D groups of experiments 3 and 4 (Tables 9 and 11). There is no significant difference in the gains of the pullets at twelve weeks between the 12.3-vitamin D groups and other groups receiving higher levels of vitamin D.

Leg Deformities at Six, Eight, and Twelve Weeks and Crooked Bones at Twelve Weeks

No rickets were observed in any of the groups of chicks in the two experiments at six weeks.

Table 12. Relation of vitamin D to rickets and other effects on chickens fed ration 2, experiment 4

International units of vitamin D from fortified cod liver oil per 100 grams of ration	Percentage of leg deformities		Percentage of the chicks that had crooked breast bones at 12 weeks	Number of chicks that died during experiment	Grams of feed required to produce one gram of gain	Mean percentage of bone ash
	rickets	slipped tendons				
0	83.3	0	61.1	6	4.80	42.82
3.1	44.4	0	38.9	6	4.67	41.84
6.2	23.5	0	23.5	4	4.17	42.74
12.3	0	5.26	0	2	3.93	48.31
25.1	0	3.03	0	10	3.83	49.91
50.2	0	2.70	0	5	3.59	50.19

Symptoms of rickets were noted in the no-vitamin D groups and the 3.1-vitamin D groups of the two experiments at eight weeks.

At twelve weeks the highest percentage of chicks with rickets in the two experiments was in the no-vitamin D groups (Tables 10 and 12), and it decreased to zero in the 12.3-vitamin D groups. Therefore, for the prevention of rickets at twelve weeks growing chicks fed ration 2 required 12.3 International units of vitamin D from fortified cod liver oil per 100 grams of feed.

The percentage of chicks with crooked breast bones in the two experiments (Tables 10 and 12) was highest in the no-vitamin D groups and decreased down to the 12.3-vitamin D group, where there were no chicks with crooked breasts. For the prevention of crooked breast bones at twelve weeks growing chicks fed ration 2 required 12.3 International units of vitamin D from fortified cod liver oil per 100 grams of ration.

Mortality, Grams of Feed Required to Produce One Gram of Gain, and Percentage of Bone Ash

The mortality of the various groups in the two experiments fed ration 2 was apparently not related to the vitamin D intake although it was rather high in all groups (Tables 10 and 12). This mortality was not unusual for chicks grown as late in the season as these chicks were.

The grams of feed required to produce one gram of gain was highest in the no-vitamin D groups of the two experiments (Tables 10 and 12), and decreased down to the 12.3-vitamin D groups; greater additions of vitamin D did not decrease the amount of feed required to produce one gram of gain. For the best utilization of the feed at twelve weeks, growing chicks fed ration 2 required a minimum of 12.3 International units of vitamin D from fortified cod liver oil per 100 grams of feed.

For complete calcification of the bones at twelve weeks the chicks in experiment 3 (Table 10) required between 25.1 and 50.2 International units of vitamin D from fortified cod liver oil per 100 grams of feed, and in experiment 4 (Table 12) they required 25.1 International units of vitamin D from fortified cod liver oil per 100 grams of feed.

Table 13. Relation of vitamin D to gain in weight of chickens fed ration 2, experiments 3 and 4 combined

Units of vitamin D from fortified cod liver oil per 100 grams of ration	Mean gain in grams					
	Cockerels			Pullets		
	Six weeks	Eight weeks	Twelve weeks	Six weeks	Eight weeks	Twelve weeks
0.0	265.0	387.1	558.0	221.3	299.4	415.1
3.1	258.8	355.4	558.2	211.1	287.5	444.8
6.2	271.0	397.9	669.1	228.7	334.3	519.4
12.3	283.7	437.8	775.5	245.0	376.2	640.9
25.1	287.7	459.7	817.2	247.6	367.4	686.9
50.2	302.7	474.9	902.9	258.9	380.3	660.3

VITAMIN D REQUIREMENTS OF CHICKENS FED RATION 3

Ration 3 (Table 2) unsupplemented and with varying amounts of No. 2 fortified cod liver oil (Table 1)—1/64 per cent, 1/32 per cent, 1/16 per cent, 1/8 per cent, and 1/4 per cent—was fed to duplicate groups of chicks in one experiment. These amounts of this fortified cod liver oil contained .4, .8, 1.6, 3.4, and 6.7 International units of vitamin D respectively per 100 grams of mixed ration as figured from the rat data of Table 1. Ration 3 was estimated to contain 7.2 International units of vitamin D equivalent per 100 grams as figured from the rat data of Table 1. The chicks on the unsupplemented ration are designated as the no-vitamin D group and other groups according to the units of vitamin D that they were receiving per 100 grams of ration from fortified cod liver oil.

Gains in Weight at Six, Eight, and Twelve Weeks

There is, with one exception, a small increase at six weeks in the gains of the cockerels and pullets of experiment 5 (Table 14) with each increase in vitamin D up to the highest amount of vitamin D fed. This exception is the pullets in the 1.6-vitamin D group whose mean gain is 6.3 grams smaller than the .8-vitamin D group. There were no significant differences

Table 14. Relation of vitamin D to gain in weight of chickens fed ration 3, experiment 5

Units of vitamin D from fortified cod liver oil per 100 grams of ration	Mean gain in grams					
	Cockerels			Pullets		
	Six weeks	Eight weeks	Twelve weeks	Six weeks	Eight weeks	Twelve weeks
0	244.1± 4.66	380.6± 7.27	527.0±16.94	190.5± 6.85	283.4±10.40	396.3±14.26
.4	246.5± 6.76	358.8± 8.75	478.2±12.89	204.5± 8.44	297.2±10.56	389.6±17.02
.8	253.0± 6.20	395.7± 8.72	538.3±15.17	228.3± 6.24	327.1± 9.26	402.9±11.67
1.6	259.8± 6.94	422.8±13.73	709.9±23.75	222.0± 7.54	362.4±11.53	568.1±18.35
3.4	263.9± 8.23	452.8±13.51	830.0±19.59	229.0± 6.01	393.7±10.15	687.3±16.82
6.7	271.0±11.28	497.7±16.59	932.0±20.45	242.3± 7.61	407.3±12.92	727.4±15.58

in the gains of the cockerels of experiment 5 (Table 14) at six weeks between the various groups, though there was a difference of 26.9 grams between the no-vitamin D group and the 6.7-vitamin D group. In the gains of the pullets at six weeks there was a significant difference between the no-vitamin D group and the .8-vitamin D group.

In the gains of the pullets at six weeks there were no significant differences between the .8-vitamin D group and other groups receiving higher levels of vitamin D in experiment 5 (Table 14). The chicks in the no-vitamin D group were not receiving adequate vitamin D for growth at six weeks.

There is a possibility that the chicks in the no-vitamin D group would not have needed any vitamin D other than that in the original ration if they had been given access to direct sunlight or if a vitamin D supplement had been included in the ration at six weeks.

It may be noted from Table 14 that the gains of the chicks at eight weeks increased with increasing amounts of vitamin D up to the highest level fed. The gains of the chicks in the 6.7-vitamin D group were larger than those of the chicks in the 3.4-vitamin D group for both cockerels and pullets, though not significantly larger in either case. The chicks in the 3.4-vitamin D group were probably not receiving enough vitamin D for the best growth at eight weeks, but this requirement might have been taken care of at a lower level than the 6.7-vitamin D group was receiving if such a level of vitamin D had been fed.

It may be noted from Table 14 that the gains of the chicks at twelve weeks increased with increasing amounts of vitamin D up to the highest level fed. The difference in the gains of the cockerels at twelve weeks in experiment 5 (Table 14) between the 3.4-vitamin D group and the 6.7-vitamin D group is significant. The difference in the gains of corresponding groups of pullets is not significant although the mean gain of the 6.7-vitamin D group is larger than that of the 3.4-vitamin D group. Higher levels of vitamin D than 6.7 International units per 100 grams of feed might have produced higher gains with chicks fed ration 3.

There is an indication that the vitamin D requirement of the cockerel in experiment 5 (Table 14) is higher than that of the pullet.

Leg Deformities at Six, Eight, and Twelve Weeks and Crooked Breast Bones at Twelve Weeks

Symptoms of rickets were noted at six weeks in the no-vitamin D group of experiment 5. The chicks in the no-vitamin D group were found to be sitting rather than standing the majority of the time. This indicates that the legs of some of the chicks in the no-vitamin D group were not as strong as they might have been. At eight weeks, it may be noted from Table 15, the percentage of chicks with rickets decreased with increasing amounts of vitamin D, and there were no rickets in the 3.4-vitamin D group. Growing chicks fed ration 3 required 3.4 International units of vitamin D from fortified cod liver oil per 100 grams of ration for the first eight weeks. At twelve weeks, there were not any chicks with rickets in

the 3.4-vitamin D group of experiment 5 (Table 15); the percentage of chicks with rickets decreased with increasing amounts of vitamin D; the percentage of chicks with rickets was considerably higher at twelve weeks than at eight weeks, showing that a larger percentage of the chicks not

Table 15. Relation of vitamin D to rickets and to other criteria on chickens fed ration 3, experiment 5

International units of vitamin D from fortified cod liver oil per 100 grams ration	Percentage of chicks with rickets at			Percentage of chicks that had slipped tendons at twelve weeks	Percentage of chicks that had crooked breast bones at twelve weeks	Number of chicks that died during the experiment	Grams of feed required to produce one gram of gain	Mean percentage of bone ash
	Eight weeks	Ten weeks	Twelve weeks					
0	83.3	91.3	100.0	0	100.0	8	4.91	37.18
.4	60.8	84.3	95.8	0	95.8	2	5.10	35.64
.8	44.2	77.6	95.7	0	100.0	1	4.87	36.93
1.6	11.4	34.9	41.5	0	51.2	9	4.40	42.88
3.4	0	0	0	2.2	6.7	5	4.06	46.70
6.7	0	0	0	0	0	5	4.00	50.27

receiving adequate vitamin D come down with rickets as they get older. For the prevention of rickets at twelve weeks growing chicks fed ration 3 require 3.4 International units of vitamin D from fortified cod liver oil per 100 grams of ration.

The percentage of chicks with crooked breast bones decreased with increasing amounts of vitamin D in experiment 5 (Table 15). There were no chicks with this deformity in the 6.7-vitamin D group. Thus for the prevention of crooked breasts at twelve weeks growing chicks fed ration 3 required 6.7 International units of vitamin D from fortified cod liver oil per 100 grams of ration.

Mortality, Grams of Feed Required to Produce One Gram of Gain, and Percentage of Bone Ash at Twelve Weeks

The number of chicks in experiment 5 (Table 15) that died during the experimental period apparently was not related to the vitamin D intake. There was a total of 312 chicks at the beginning of the experiment and only 30 died during the twelve-week period.

Between the no-vitamin D group and the .8-vitamin D group there is very little difference in the grams of feed required to produce one gram of gain. There is a significant difference in the grams of feed required to produce one gram of gain between the .8-vitamin D group and the 1.6-vitamin D group and between the 1.6-vitamin D group and the 3.4-vitamin D group, but very little difference in the grams of feed required to produce one gram of gain between the 3.4-vitamin D group and the 6.7-vitamin D group. The chicks in the 6.7-vitamin D group which were receiving the highest level of vitamin D in this experiment required more grams of feed to produce one gram of gain than was required in the best

groups of chicks fed rations 1 and 2, and it may be possible that the chicks in the 6.7-vitamin D group were not receiving adequate vitamin D for the best utilization of the feed.

There is very little difference in the percentage of bone ash between the no-vitamin D group and the .8-vitamin D group, but in the groups fed higher levels of vitamin D the percentage of bone ash increases with each additional increase of vitamin D up to the highest level fed in the 6.7-vitamin D group. It is quite possible that the 6.7-vitamin D group was receiving adequate vitamin D for complete calcification of the bones, since the percentage of bone ash in this group is as high as any reported in this bulletin, and in other experiments levels of vitamin D as high as 50.2 units of vitamin D per 100 grams of ration from fortified cod liver oil were fed. For complete calcification of the bones growing chicks fed ration 3 required a minimum of 6.7 International units of vitamin D from fortified cod liver oil per 100 grams of feed.

VITAMIN D REQUIREMENTS OF CHICKENS FED RATION 4

Ration 4 (Table 2) unsupplemented and with varying amounts of cod liver oil No. 2 (Table 1)—1/64 per cent, 1/32 per cent, 1/16 per cent, 1/8 per cent, and 1/4 per cent—was fed to duplicate groups of chicks in one experiment. These amounts of cod liver oil contained .4, .8, 1.6, 3.4, and 6.7 International units of vitamin D respectively per 100 grams of mixed ration as figured from the rat data of Table 1. Ration 4 was estimated to contain 6.7 International units of vitamin D equivalent per 100 grams as figured from the rat data of Table 1. The chicks on the unsupplemented ration are designated as the no-vitamin D group, and other groups according to the units of vitamin D that they are receiving from cod liver oil.

Gains in Weight at Six, Eight, and Twelve Weeks

There is a significant difference at six weeks in the gains of both cockerels and pullets of experiment 6 (Table 16) between the no-vitamin D group and the .8-vitamin D group. The gains of the chicks in some of the groups receiving higher levels than the .8-vitamin D group are higher than those of this group, but with the exception of two cases these differences are within the error of the difference. The chickens in the .8-vitamin D group were receiving sufficient vitamin D for growth at six weeks.

There is an increase in the gains of the cockerels at eight weeks in experiment 6 (Table 16) with each increase of vitamin D up to the highest level fed. However, the difference between the 3.4-vitamin D group and 6.7-vitamin D group is not significant. There is an increase in the gains of the pullets of experiment 6 (Table 16) at eight weeks with each increase in vitamin D up to the 3.4-vitamin D group, and there is very little difference between the 3.4-vitamin D group and the 6.7-vitamin D group. The chicks in the 3.4-vitamin D group of experiment 6 were receiving sufficient vitamin D for growth at eight weeks.

With the exception of the 1.6-vitamin D group there is an increase in the gains of the cockerels in experiment 6 (Table 16) at twelve weeks with each increase of vitamin D up to the highest level of vitamin D fed. The difference between the 3.4-vitamin D group and the 6.7-vitamin D group (Table 16) is not significant, and it may be noted from Table 16 that the

Table 16. Relation of vitamin D in feed to gain in weight of chickens fed ration 4, experiment 6

International units of vitamin D from fortified cod liver oil per 100 grams of ration	Mean gain in grams					
	Cockerels			Pullets		
	Six weeks	Eight weeks	Twelve weeks	Six weeks	Eight weeks	Twelve weeks
0	241.9± 5.72	320.7± 8.90	430.3±17.44	213.6± 4.90	273.2± 7.81	340.3±12.99
.4	260.1± 4.17	344.9± 7.50	518.7±13.93	225.5± 4.35	289.6± 8.26	400.5±14.32
.8	270.3± 3.26	390.2± 6.52	614.8±14.20	239.8± 3.68	300.3± 8.90	418.3±16.96
1.6	237.9± 3.49	346.7± 6.68	572.3±18.60	216.8± 7.91	316.5±16.02	502.2±26.28
3.4	277.3± 4.05	433.9± 6.01	785.8±11.34	264.4± 3.94	397.9± 7.81	690.1±10.15
6.7	287.7± 4.32	459.4± 7.92	815.9±16.09	251.6± 5.50	391.6± 8.38	657.5±17.70

difference in the gains of the cockerels at twelve weeks between the 3.4-vitamin D group and the 6.7-vitamin D group is but very little larger than it was at eight weeks. The gains of the pullets at twelve weeks in experiment 6 (Table 16) increase with each increasing amount of vitamin D up to the 3.4-vitamin D group. The gains of the pullets in the 6.7-vitamin D group are lower than those of the 3.4-vitamin D group.

Leg Deformities at Six, Eight, Ten, and Twelve Weeks and Crooked Breast Bones at Twelve Weeks

There were 28.6 per cent of the chicks in the no-vitamin D group of experiment 6 (Table 17) that showed visible signs of rickets at six weeks. No signs of rickets were noted in any of the groups receiving vitamin D from cod liver oil at this time. At eight weeks 64.0 per cent of the chicks in the no-vitamin D group and 52.1 per cent of the chicks in the .4-vitamin D group (Table 17) showed signs of rickets. There was a higher percentage of rickets in the 1.6-vitamin D group than in the .8-vitamin D group. At ten and twelve weeks (Table 17) the percentage of chicks with rickets decreased with increasing amounts of vitamin D up to the 3.4-vitamin D group, where no symptoms of rickets were noted. It may be seen from Table 17 that there is a definite increase in the percentage of chicks with rickets in each group as the chicks grew older, showing that the need for vitamin D was increasing with the increasing age of the chicks.

At twelve weeks the percentage of chicks with crooked breast bones in experiment 6 (Table 17) was highest in the no-vitamin D group and decreased down to the 3.4-vitamin D group, where no cases of this deformity were noted.

Mortality, Grams of Feed Required to Produce One Gram of Gain, and Percentage of Bone Ash

The mortality of the chicks in experiment 6 (Table 17) was small and apparently was not related to the vitamin D intake.

The grams of feed required to produce one gram of gain in experiment 6 (Table 17) decreased with increasing amounts of vitamin D up to the 3.4-vitamin D group, and there was very little difference in the grams of feed required to produce one gram of gain between the 3.4-vitamin D group and the 6.7-vitamin D group.

The mean percentage of bone ash in experiment 6 (Table 17) increases with increasing amounts of vitamin D up to the highest amount fed, and

Table 17. Relation of vitamin D to rickets and to other criteria on chickens fed ration 4, experiment 6

International units of vitamin D from fortified cod cod liver oil per 100 grams ration	Percentage of chicks with rickets at				Percentage of chicks that had slipped tendons at twelve weeks	Percentage of chicks that had crooked breast bones at twelve weeks	Number of chicks that died during the experiment	Grams of feed required to produce one gram of gain	Mean percentage of bone ash
	Six weeks	Eight weeks	Ten weeks	Twelve weeks					
0	28.6	64.0	80.0	85.7	0	87.8	2	5.01	38.26
.4	0	52.1	68.9	90.9	0	84.1	7	4.76	38.55
.8	0	9.3	47.6	63.4	0	65.9	8	4.47	42.42
1.6	0	22.9	45.5	50.0	0	45.5	7	4.41	44.37
3.4	0	0	0	0	0	0	7	3.89	48.50
6.7	0	0	0	0	0	0	4	3.93	50.06

it cannot be said that the bones of the chicks in the 6.7-vitamin D group are completely calcified since a higher level of vitamin D than this was not fed. However, the mean percentage of bone ash of the 6.7-vitamin D group in experiment 6 (Table 17) compares very favorably with the highest mean percentages of bone ash obtained in previous experiments reported in this bulletin, where levels of vitamin D as high as 50.2 International units per 100 grams of feed were fed.

DISCUSSION

The vitamin D requirements of the chicks reported in this bulletin varied with the different rations. These rations varied widely in their calcium content. There is a possibility that rations 1, 3, and 4 may be fed up to six weeks without any additional vitamin D provided the chicks are given access to direct sunlight or a vitamin D supplement is included in the ration after this time. The vitamin D requirements of the chicks fed ration 2 were apparently higher than those of chicks receiving rations 1, 3, or 4. The minimum vitamin D requirements of growing chicks fed ration 1 may be estimated at 12.3 International units of vitamin D from fortified cod liver oil per 100 grams of feed. The increased gains secured by the above addition of vitamin D would more than pay for the vitamin D supplement needed to make this addition. Growing chicks fed ration 2 required 50.2 International units of vitamin D from fortified cod liver oil

per 100 grams of feed. This amount was required for greatest growth and complete calcification of the bones at twelve weeks, but was more than was needed for the prevention of rickets and crooked breast bones. The reason for this higher vitamin D requirement is the low calcium content of the second ration. This deficiency is attributed to a different grade of meat and bone scrap and it brings out the fact that one should use only the best grades of feed, for a good formula might be ruined by the use of an inferior grade of feed. Ration 3 is the same as ration 2 except for a different grade of meat and bone scrap which increased the calcium content. This lowered the vitamin D requirements of this ration. The minimum vitamin D requirements of ration 3 may be estimated to be approximately 6.7 International units of vitamin D from fortified cod liver oil per 100 grams of feed. This was the highest level of vitamin D fed to the chicks that received ration 3, and it is possible that a higher level of vitamin D would have produced better results than were secured. However, the gains made by the chicks in the 6.7-vitamin D group fed ration 3 and their mean percentage of bone ash compare favorably with that of chicks fed the other rations which received much higher levels of vitamin D. The minimum vitamin D requirements of ration 4 may be estimated to be approximately 6.7 International units of vitamin D from fortified cod liver oil per 100 grams of feed. This was the highest level of vitamin D fed to the chicks that received ration 4. There was very little difference in the gains of the chicks in the 3.4-vitamin D group and the 6.7-vitamin D group. The percentage of bone ash was higher in the 6.7-vitamin D group than in the 3.4-vitamin D group, experiment 6 (Table 17). The gains of the chicks in the 6.7-vitamin D group of experiment 6 (Table 16) are lower on the average at twelve weeks than the highest gains of the other experiments. Therefore, it is possible that a higher level of vitamin D than that fed in experiment 6 would have produced higher gains than were secured, although the mean percentage of bone ash of the 6.7-vitamin D group of experiment 6 (Table 17) compares favorably with the highest mean percentage of bone ash secured with higher levels of vitamin D in other experiments reported in this bulletin.

There is an indication from the data presented in this bulletin that the requirement for vitamin D of the cockerels is higher than that of the pullets. Such an indication is quite plausible since the sexual development of the cockerels takes place earlier than that of the pullets.

The low calcium content of ration 2 probably caused the larger vitamin D requirements of this ration over that of the other rations fed.

The increase in the phosphorus content of rations 3 and 4 probably explains why these rations contain less vitamin D equivalent than rations 1 and 2. These conclusions are in agreement with the work of Bethke, Steenbock, and Nelson (4), Karelitz and Shohl (16), Sherman and Stiebeling (29), and Holmes and Pigott (15), in that there is a definite relationship between the ratio and the amounts of calcium and phosphorus in the feed and the need for vitamin D.

Growing chickens fed ration 1 required approximately 12.3 International units of vitamin D from cod liver oil per 100 grams of feed, which is

slightly lower than that reported by Bethke, Record, and Kennard (3), Carver, Robertson, Brazie, Johnson, and St. John (7), Murphy, Hunter, and Knandel (22), and Russell, Taylor, and Wilcox (24). Bethke and coworkers (3) fed 10.8 and 18.9 International units of vitamin D from cod liver oil per 100 grams of feed and concluded that 18.9 units was the correct amount; Carver and coworkers (7) fed 8.4 and 16.8 International units of vitamin D from cod liver oil per 100 grams of feed and concluded that 16.8 units was the right amount; Murphy et al. (22) fed 8.5 and 17.0 International units of vitamin D from cod liver oil per 100 grams of feed and concluded that 17 units was the correct amount; and the lowest level fed by Russell, Taylor, and Wilcox (24) was 14.9 International units of vitamin D from cod liver oil per 100 grams of feed, which they concluded was the minimum vitamin D requirement of chickens up to 8 weeks of age. If these workers had fed other levels of vitamin D slightly lower than their estimated requirements it is possible that their estimations might have been somewhat lower. Their results, therefore, are not contradictory to the results here reported. The vitamin D requirements of chickens fed ration 2 were approximately 4 times those found for chickens fed ration 1, probably because of the low calcium content of ration 2. It cannot be said that the vitamin D supplied to chickens fed rations 3 and 4 was adequate, since higher levels than that fed with these rations might have produced better results than those secured in experiments 5 and 6.

The number of units of vitamin D required by growing chickens appears to depend upon the nature of the ration, especially the percentages of

Table 18. Summary of the requirements of vitamin D for chickens grown in the absence of sunlight

Experiment Number	Percentage of calcium in ration	Percentage of phosphorus in ration	International units of vitamin D from fortified cod liver oil required for					
			Maximum growth		Prevention of		Best utilization of the feed	Calcification of the bones
			Cockerels	Pullets	Rickets	Crooked breast bones		
1	1.48	0.65	12.3	12.3	3.1	3.1	12.3	3.1
2	1.48	0.65	12.3	12.3	3.1	3.1	12.3	3.1
3	0.96	0.66	50.2	25.1	12.3	12.3	12.3	50.2
4	0.96	0.66	50.2	50.2	12.3	12.3	50.2	25.1
5	1.26	0.77	6.7	6.7	3.4	6.7	6.7	6.7
6	1.36	0.78	6.7	3.4	3.4	3.4	3.4	6.7

calcium and phosphorus contained in it, and upon the particular method used in ascertaining whether or not vitamin D is deficient. A comparison of these factors is given in Table 18, which is a summary of the various tables already given. It is evident from the table the quantity of vitamin D required to prevent rickets or crooked breast bones is, as a rule, much less than that required to produce the maximum growth of the chickens. The conclusions arrived at from considering rickets alone or crooked breasts alone are consistently much lower than those reached from con-

sidering the maximum gains of the chickens. In the first four experiments it takes about four times as much vitamin D to produce a maximum gain as to prevent rickets or crooked breast bones. In the last two experiments it takes about half as much vitamin D to prevent rickets or crooked breast bones as it does to produce maximum growth. In the first two experiments the percentages of bone ash agree with the requirements for the prevention of rickets and crooked breast bones, but in the last four experiments they more nearly correspond with the gains of the chickens. The utilization of the feed is in line with the gains of the chickens.

By any of the methods used it is seen that the vitamin D requirements vary to a considerable extent with the nature of the ration being fed. They are lowest in the last two experiments, which differ to a considerable extent in their content of calcium and phosphorus but do not contain as large a percentage of calcium as the experiments of which the requirements are intermediate.

SUMMARY AND CONCLUSIONS

1. Four rations which differed somewhat in their ingredients so as to represent different feeds and which differed in their calcium and phosphorus content were fed to chickens.

2. The rations and cod liver oils used were analyzed for vitamin D by the lime test.

3. For the first six weeks even in the absence of sunlight chickens fed a ration with the proper amounts of calcium and phosphorus apparently need no additional vitamin D to make good growth.

4. The vitamin D requirement of the cockerel appears to be higher than that of the pullet.

5. The vitamin D requirements of chickens vary with the particular factor taken into consideration in deciding upon the effect of the vitamin D supplied. Gain in weight required more vitamin D than any one of the other factors studied.

6. The number of units of vitamin D required by growing chickens appears to depend upon the nature of the ration, especially the percentages of calcium and phosphorus contained in it.

7. Chickens fed a ration containing 1.48 per cent calcium and 0.65 per cent phosphorus required 12.3 International units of vitamin D from cod liver oil per 100 grams of feed for maximum growth and best utilization of the feed, and 3.1 International units of vitamin D from cod liver oil per 100 grams of feed for the prevention of rickets and crooked breast bones and the calcification of the bones at twelve weeks.

8. Chickens fed a ration containing 0.96 per cent calcium and 0.66 per cent phosphorus required up to twelve weeks 50.2 International units of vitamin D from cod liver oil per 100 grams of feed for maximum growth, best utilization of the feed, and calcification of the bones, and 12.3 International units of vitamin D from cod liver oil per 100 grams of feed for the prevention of rickets and crooked breast bones. This high vitamin D requirement was probably due to the low calcium content of this ration.

9. Chickens fed a ration containing 1.26 per cent calcium and 0.77 per cent phosphorus required up to 12 weeks 6.7 International units of vitamin D from cod liver oil per 100 grams of feed for maximum growth, prevention of crooked breast bones, best utilization of the feed, and calcification of the bones, and 3.4 International units of vitamin D from cod liver oil per 100 grams of ration for the prevention of rickets. The maximum level of vitamin D may not have been fed with this ration.

10. Chickens fed a ration containing 1.36 per cent calcium and 0.78 per cent phosphorus required up to twelve weeks 6.7 International units of vitamin D from cod liver oil per 100 grams of feed for maximum growth and calcification of the bones, and 3.4 International units of vitamin D from cod liver oil per 100 grams of feed for the prevention of rickets and crooked breast bones and for the best utilization of the feed. The maximum level of vitamin D may not have been fed with this ration.

BIBLIOGRAPHY

1. Bethke, R. M., and Kennard, D. C.
1924-25. Rearing Chicks to Maturity Indoors. Poultry Sci. 4: 74-78.
2. Bethke, R. M., Kennard, D. C, Kick, C. H., and Zinzalian, G.
1929. The Calcium-Phosphorus Relationship in the Nutrition of the Growing Chick. Poultry Sci. 8: 257-65.
3. Bethke, R. M., Record, P. R., and Kennard, D. C.
1933. The Comparative Antirachitic Efficiency of Irradiated Ergosterol, Irradiated Yeast, and Cod Liver Oil for Chickens. J. Nutrition 6: 413-25.
4. Bethke, R. M., Steenbock, H., and Nelson, M. T.
1923. Fat Soluble Vitamins. XV. Calcium and Phosphorus Relations to Growth and Composition of Blood and Bone with Varying Vitamin Intake. J. Biol. Chem. 58: 71-103.
5. Bills, C. E., Honeywell, E. M., Wirick, A. M., and Nussmeier, M. J.
1931. A Critique of the Line Test for Vitamin D. J. Biol. Chem. 90: 619-36.
6. Brown, H. B., and Shohl, A. T.
1930. Rickets in Rats. XI. The Alteration of Calcium and Phosphorus Metabolism of Normal and Ricketic Rats Produced by Irradiated Ergosterol. J. Biol. Chem. 86: 245-62.
7. Carver, J. S., Robertson, E. I., Brazie, D., Johnson, R. H., and St. John, J. L.
1934. The Vitamin D Requirements of Chickens. Bull. 299, Washington Agri. Exp. Station.
8. Drummond, J. C.
1916. Biochem, J. 10: 77.
9. Emmett, A. D., and Peacock, G.
1923. Does the Chick Require the Fat Soluble Vitamins? J. Biol. Chem. 56: 679-93.

10. Halvorson, H. A., and Lachat, L. L.
1934. Antirachitic Activity of Vitamin D Supplements for Poultry. Minn. Div. of Feed and Fertilizer Control Report.

11. Hart, E. B., Halpin, J. G., and Steenbock, H.
1920. Use of Synthetic Diets in the Growth of Baby Chicks. A Study of Leg Weakness in Chickens. J. Biol. Chem. 43: 421-42.

12. Hart, E. B., Halpin, J. G., and Steenbock, H.
1922. The Nutritional Requirements of Baby Chicks. II. Further Study of Leg Weakness in Chickens. J. Biol. Chem. 52: 379-86.

13. Hart, E. B., Steenbock, H., and Lepkovsky, S., and Halpin, J. G.
1923. The Nutritional Requirements of Baby Chicks. III. The Relation of Light to the Growth of the Chicken. J. Biol. Chem. 58: 33-44.

14. Hart, E. B., and Scott, H. T., and Kline, O. L., and Halpin, J. G.
1930. The Calcium-Phosphorus Ratio in the Nutrition of Growing Chicks. Poultry Sci. 9: 296-306.

15. Holmes, A. D., and Pigott, M. G.
1931. Effect of Cod Liver Oil on Calcium Metabolism of Young Chicks. Ind. and Eng. Chem. 23: 190-96.

16. Karelitz, S., and Shohl, A. T.
1927. Rickets in Rats. II. The Effect of Phosphate Added to the Diet of Ricketic Rats. J. Biol. Chem. 73: 665-77.

17. McCollum, E. V., and Simmonds, N., and Shipley, P. G., and Park, E. A.
1922. Studies on Experimental Rickets. XVI. A Delicate Test for Calcium Depositing Substances. J. Biol. Chem. 51: 41-49.

18. McCollum, E. V., Simmonds, N. Shipley, P. G., and Park, E. A.
1922. Studies on Experimental Rickets. XII. Is There a Substance Other Than Fat-Soluble A Associated with Certain Fats Which Plays an Important Role in Bone Development? J. Biol. Chem. 50: 5-30.

19. Mellanby, E.
1919. An Experimental Investigation of Rickets. Lancet 1, 407-12.

20. Moritz, A. R., and Krenz, C.
1929-30. The Relation of the Fat Soluble Vitamins (A and D) to the Development of Experimental Rickets in Rabbits. J. Nutrition 2: 257-64.

21. Munch, J. C.
1931. Bioassays—A Handbook of Quantitative Pharmacology. 842-46. The Williams and Wilkins Company, Baltimore.

22. Murphy, R. R., Hunter, J. E., and Knandel, H. C.
1934. The Vitamin D Requirements of Growing Chicks and Laying Hens. Bulletin 303, Penn. Agri. Exp. Station.

23. Osborne, T. B., and Mendel, L. B.
1918. The Growth of Chicks in Confinement. J. Biol. Chem. 33: 433-38.

24. Russell, Walter C., Taylor, M. W., and Wilcox, D. E.
1934. The Fate of the Antirachitic Factor in the Chicken. III. The Effective Levels and the Distribution of the Factor From Cod Liver Oil and From Irradiated Ergosterol in Certain Tissues of the Chicken. J. Biol. Chem. 107: 735-46.

25. Steenbock, H., and Black, A.
1925. Fat-Soluble Vitamins. XVIII. The Induction of Growth-Promoting and Calcifying Properties in Fats and Their Unsaponifiable Constituents by Exposure to Light. J. Biol. Chem. 64: 263-98.

26. Steenbock, H., and Kletzien, S. W. F., and Halpin, J. G.
1932. The Reaction of the Chicken to Irradiated Ergosterol and Irradiated Yeast as Contrasted with the Natural Vitamin D of Fish Liver Oils. J. Biol. Chem. 97: 249-64.

27. Sherwood, R. M.
1932. Lime and Phosphoric Acid Requirements for Chicks. Bulletin 462 Texas Agri. Exp. Station.

28. Sherwood, R. M., and Couch, J. R.
1933. Feeding for Efficient Growth and Prevention of Slipped Tendons in Chickens. Bulletin 476, Texas Agri. Exp. Station.

29. Sherman, H. C., and Stiebeling, H. K.
1930. The Relation of Vitamin D to Deposition of Calcium in Bone. Soc. Exp. Biol. and Med. Proc. 27: 663.

30. Wilgus, H. S., Jr.
1931. The Quantitative Requirement of the Growing Chick for Calcium and Phosphorus. Poultry Sci. 8: 107-18.