THE COMPARATIVE VALUE OF COD-LIVER OIL ALONE AND IN COMBINATION WITH IRRADIATED ERGOSTEROL AS A SOURCE OF VITAMIN D FOR CONFINED LAYING HENS [1]

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SURVEY OF LITERATURE

Cod-liver oil has been used for many centuries as an antirachitic agent (*10*).[2] In 1922 McCollum, Simmonds, Shipley, and Park (*24, p. 7*) published an account of a newly discovered factor in cod-liver oil, separate from vitamin A, "which exerts a directive influence on the bone development and enables animals to develop on an inadequate supply of calcium much better than they could otherwise do." Four years later Holmes, Brown, Smith, Treadwell, and Whitelock (*18*) pointed out that cod-liver oil in the diet of laying hens increased egg production and hatchability. That the newly discovered vitamin D was a major factor in their results can be gathered from the work of Hughes, Payne, and Latshaw (*20*), who had previously obtained the same results with ultraviolet irradiation.

In 1924 Steenbock and Black (*31*) found that diets for rats could be made antirachitic and growth promoting by irradiation with a quartz mercury-vapor lamp. In the same year Hess (*13*) and Hess and Weinstock (*15*) activated linseed oil and cottonseed oil by means of irradiation. Either of these activated oils when added to a rickets-producing diet was able to protect rats from rickets. Hess and Weinstock also found that by irradiating cod-liver oil they could enhance its antirachitic value.

Since the unsaponifiable portion of fat contains a large quantity of cholesterol, for a time many research workers considered cholesterol as the precursor of vitamin D. However, Heilbron, Kamm, and Morton (*12*), Hess and Windaus (*16*), and Bills, Honeywell, and MacNair (*6*) demonstrated that in the nutrition of rats, the vitamin D precursor is ergosterol, which is present as a minute impurity in the cholesterol. From this irradiated sterol a crystalline "synthetic" vitamin D was finally isolated. Dissolved in corn oil, irradiated ergosterol is being used extensively as a source of vitamin D.

In the nutrition of rats, Russell, Taylor, and Wilcox (*30*) found that irradiated ergosterol was as efficacious in preventing rickets as cod-liver oil when the two were fed in the approximate equivalent rat-unit dosage. However, in human nutrition, De Sanctis and Craig (*8*) and Barnes, Brady, and James (*1*) concluded that irradiated ergosterol was not so satisfactory an agent in the prevention of rickets as cod-liver oil in the approximate equivalent rat-unit dosage. Holmes (*17, p. 214*), after making a comprehensive review of the

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[2] Reference is made by number (italic) to Literature Cited, p. 218.

literature, decided that as an antirachitic agent "either vitamin D as it occurs in cod-liver oil possesses properties not present in irradiated ergosterol, or that the vitamin A or some other substance in cod-liver oil enhances the antirachitic vitamin in the oil * * *." In the bone development and growth of chicks, Mussehl and Ackerson (*27*), Massengale and Bills (*25*), Bethke, Record, and Kennard (*2*), and Massengale and Nussmeier (*26*) also found that activated ergosterol was much less effective than the rat-equivalent quantity of cod-liver oil. Bethke and associates estimated that 15 to 20 times as many rat units of vitamin D per unit of feed, in the form of irradiated ergosterol, is required for normal bone formation, as in the form of cod-liver oil. Finally, in the feeding of mature hens for optimum egg production and hatchability, Branion and Smith (*7*), Russell, Taylor, and Wilcox (*29*), Bethke, Record, Kick, and Kennard (*3*), and Titus and Nestler (*33*) showed that irradiated ergosterol gave poorer results than the rat-equivalent dosage of cod-liver oil.

Titus and Nestler found that cod-liver oil fed at high levels had a markedly deleterious effect on both egg production and hatchability. They suggested that the optimum quantity of cod-liver oil for strictly confined laying hens is between 1 and 2 percent of the diet, and that when irradiated ergosterol is used as a source of vitamin D, 10 to 20 times as much of this vitamin (as measured by assays with rats) must be fed.

Barnes and associates (*1*), in their observations on humans, found that there was no evidence of superiority among a group of children receiving a mixture of cod-liver oil and irradiated ergosterol as compared with a group given cod-liver oil only. Bethke and associates (*2*) concluded from their work with chicks that a mixture of cod-liver oil and irradiated ergosterol is less effective than the rat-equivalent quantity of cod-liver oil only for proper bone calcification.

Holmes and Pigott (*19*) determined on rats the antirachitic activity of six samples representing five brands of irradiated ergosterol 100 D and found that these samples did not possess uniform antirachitic value. A sample of irradiated ergosterol 100 D that possessed the highest activity of those listed had less than 60 times the antirachitic activity of the best cod-liver oil tested.

Rider, Sperti, Goode, and Cassidy (*28, p. 456*), after making a comprehensive review of the literature, suggested that—

> the expected and the actual clinical potencies of irradiated ergosterol may have been accounted for in part by the now overwhelmingly evident fact that irradiation of ergosterol with the full ultraviolet of the quartz mercury arc produces a multiplicity of products. Some of these products are known to be inactive physiologically and others to be toxic without appreciable antirachitic activity.

These workers found that the discrepancies between the activities of antirachitic agents in rats and chickens are to some extent paralleled by similar discrepancies between their activities in rats and children. In the absence of vitamin D the chicken develops rickets in spite of an optimum calcium and phosphorus ratio and content in the diet. To produce rickets in the rat the diet must be definitely unbalanced with respect to these two constituents. Hess, Lewis, and Rivkin (*14, p. 1888*) make the observation that—

> there is an essential difference in the pathogenesis of rickets in the rat and in infants, that the former is regularly brought about simply by a lack of phosphorus in the diet, whereas rickets never comes about in infants as a result of such a deficiency * * *.

When Bills and associates (*6*) in 1928 activated by ultraviolet irradiation the ergosterol that they obtained from cholesterol, they noted that they could also activate the ergosterol-free cholesterol by the same irradiation. The following year Koch, Koch, and Lemon (*22*) and Koch, Koch, and Ragins (*23*) concluded that provitamin D activity is not limited to ergosterol, but that it may be a general property in varying degrees of various sterols or certain forms of these sterols. More recently Waddell (*34*), Koch and Koch (*21*), Hathaway and Lobb (*11*), and Bethke, Record, and Wilder (*4*) found that in chicken nutrition the vitamin D of irradiated, heated, and purified cholesterol has properties resembling more closely the natural vitamin D of cod-liver oil than has the vitamin D of irradiated ergosterol. In 1937, Bills (*5*) pointed out that at least eight forms of vitamin D have been artificially prepared, and at least two forms occur in fish oils.

In view of the fact that the chicken is less sensitive than the rat to the vitamin D produced by irradiating ergosterol, whereas the opposite is true of the vitamin D in cod-liver oil, the question arises: Is a mixture of vitamin D from these two sources more effective in the case of laying hens than the vitamin D from cod-liver oil alone? As a result of an experiment originally planned for a study of the effect of irradiated ergosterol on the transfer of vitamin A to eggs (DeVaney, Titus, and Nestler (*9*)), data have been accumulated which throw some light on this question. The present paper deals with the writer's analysis of these data.

EXPERIMENTAL METHODS AND MATERIALS

The experiment reported in this paper was conducted at the Agricultural Research Center, Beltsville, Md., during the 48-week period beginning August 29, 1933, and ended July 31, 1934. Six pens were used in this study. They were approximately 8 by 11 feet in size and were located on the second floor of a frame building. Ordinary window glass filtered all the sunlight that entered.

In this experiment there were used 108 cross-bred pullets, the progeny of a mating of Rhode Island Red males with Barred Plymouth Rock females. These birds were distributed in the 6 pens at random, 18 to a pen. Two Rhode Island Red cockerels were placed in each pen, but 5 weeks later the poorer one of each pair was removed. To insure uniform fertility the males were transferred from one pen to the next twice a week.

Throughout the experiment the birds were allowed all they would eat of the following all-mash diet:

	Percent
Ground yellow corn	38.5
Pure wheat bran	18.8
Rolled oats	11.6
Alfalfa leaf meal	4.2
Desiccated meat meal	8.0
North Atlantic fish meal	7.0
Dried buttermilk	5.0
Ground limestone	2.9
Special steamed bonemeal	3.0
Anhydrous sodium sulphate	.5
Sodium chloride	.5
Total	100.0

The proximate chemical analysis of this diet was as follows:

	Percent
Moisture	10.0
True protein [1]	19.2
Nonprotein nitrogen compounds	1.7
Ash	10.3
Fat	4.4
Fiber	4.1
Nitrogen-free extract	50.3
Total	100.0

[1] The diet contained 21.5 percent of crude protein.

A phosphorus level of 1.2 percent and a calcium level of 3.0 percent were maintained throughout the experiment.

The following oil mixtures were added to the diet: Mixture of (1) 0.5 percent of corn oil and (2) 1 percent of cod-liver oil for pen 1, 2 percent of cod-liver oil for pen 2, and 8 percent of cod-liver oil for pen 3; and a mixture of (1) 0.5 percent of irradiated ergosterol 160 D and (2) 1 percent of cod-liver oil for pen 1A, 2 percent of cod-liver oil for pen 2A, and 8 percent of cod-liver oil for pen 3A.

The rachitically inert corn oil was used merely to make the fat content of the diets fed in the first three pens correspond with that of the diets fed in the last three pens. On the basis of the conclusion drawn by Titus and Nestler (*33*), 0.5 percent of irradiated ergosterol 160 D may be considered equivalent to 4 percent of cod-liver oil in its vitamin D value to laying hens. Therefore, whereas pens 1, 2, and 3 received 1, 2, and 8 percent of cod-liver oil, respectively, pens 1A, 2A, and 3A received the equivalent of 5, 6, and 12 percent of cod-liver oil, respectively.

The weights of the birds were obtained at the beginning of the experiment and at the end of every 4-week period thereafter. A record was kept of the weight of the feed given to the birds and the weight of the residue at the end of each 4-week period. Four times a day throughout the experiment the birds in the trap nests were removed and the eggs were collected. Every 4 weeks all the sound eggs laid during a period of 10 days were incubated in order to obtain data on fertility and hatchability. During thirty-three 5-day periods, systematically scheduled throughout the year, all eggs were weighed; and during every third 5-day period the eggs, in addition to being weighed, were tested for shell strength by the method of Swenson and James (*32*), as modified by Titus and Nestler (*33*). In this test for eggshell strength a steel ball weighing 3.5282 g was dropped from various heights through a tube on each egg, which was held in place under the end of the tube. During the last half of the experiment studies were made of the weight and thickness of the eggshells, thickness of the membranes of the eggshells, weight and color index of the yolks, and the weight of the whole and thick albumen, from eggs obtained during five 5-day periods 4 weeks apart. The thickness of the eggshells and the thickness of the shell membranes were determined with micrometer calipers immediately after the eggs were broken. The two membranes were measured together. A color scale designed by Dr. Paul F. Sharp, of Cornell University, was used in the study of the color of the egg yolks. The thick albumen was sepa-

rated from the whole albumen by the use of a perforated sieve 4⅝ inches in diameter, having 64 holes per square inch, each hole being 0.081 inch in diameter.

RESULTS AND DISCUSSION

A summary of the data obtained is presented in table 1, and the statistical significance of the differences found in table 1 is presented in table 2. Most of the data were analyzed by variance analysis.

The birds in pen 1, which received 1 percent of cod-liver oil as the source of vitamin D, made significantly greater gains in live weight between August 29, 1933, and February 13, 1934—the date of maximum gains during the entire experiment—than the birds in pens 1A, 2, or 2A. The differences between the gain in live weight of the birds in pen 1 and the gains of the birds in pens 3 and 3A were large but not statistically significant. Likewise, differences between the gains in live weight of birds in any two of the pens other than pen 1 were not statistically significant.

The birds in pen 1 consumed a greater quantity of feed than any other birds. This fact accounts for their greater gains in live weight. The difference in feed consumption was statistically significant in all cases, except between the birds in pen 1 and those in pen 2A. The diets fed in pens 1A, 2, and 2A all caused significantly greater feed consumption than those fed in pens 3 and 3A. There were no statistically significant differences between the quantities of feed consumed by the birds in pens 2 and 3, which received 2 and 8 percent of cod-liver oil, respectively, as the sole source of vitamin D, and the quantities of feed consumed by the birds in pens 2A and 3A, which received corresponding levels of cod-liver oil mixed with irradiated ergosterol.

The hatchability of the eggs from either pen 1, 1A, 2, or 2A was higher than that of the eggs from either pen 3 or 3A, the difference being statistically very significant. There was no statistically significant difference between the hatchability of the eggs from a pen receiving any particular level of cod-liver oil without irradiated ergosterol and that of eggs from a pen receiving a corresponding level of cod-liver oil with irradiated ergosterol.

The production and the total weight of eggs in either pen 1, 1A, 2, or 2A also were greater than the production and the total weight of eggs in either pen 3 or 3A. However, it will be noted from table 2 that the difference between the egg production in either pen 1 or 1A and that in either pen 3 or 3A was not so statistically significant as the difference between the egg production in either pen 2 or 2A and that in either pen 3 or 3A. In the case of both egg production and the total weight of the eggs produced, there was a statistically greater significant difference between either pen 1, 1A, 2, or 2A and pen 3A than between either pen 1, 1A, 2, or 2A and pen 3. Although there was a tendency for cod-liver oil to give better results than a mixture of cod-liver oil and irradiated ergosterol, none of the differences between the results obtained at the same level of cod-liver-oil intake were statistically significant.

The only statistically significant differences found in the data for the average weight of the eggs was between either pen 1 or 1A and either pen 3 or 3A. The weights of the eggs from the former pens were heavier than those of the eggs from the latter pens.

TABLE 1.—*Effect of cod-liver oil at various levels, fed alone or mixed with irradiated ergosterol, on laying hens and their egg production*[1]

DATA THAT SHOW STATISTICALLY SIGNIFICANT DIFFERENCES[2]

Source of vitamin D	Pen no.	Birds at—		Average gain in live weight Aug. 29, 1933, to Feb. 13, 1934[3]	Average feed consumed per bird per 4-week period[4]	Average hatchability of fertile eggs[5]	Average egg production per bird per 308.5 bird days[6]	Total weight of eggs per bird per 313.8 bird days[7]	Average egg weight	Average yolk weight	Average reading of Sharp's yolk color scale
		Beginning of experiment	End of experiment								
		Number	*Number*	*Grams*	*Kilograms*	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*	
1 percent of cod-liver oil	1	18	11	679.2±53.7	4.27± .10	68.0±4.5	42.11±3.53	7,985±672	58.0±1.0	19.1± .4	62±6
1 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	1A	18	14	510.3±47.0	4.00± .10	64.4±4.0	42.98±3.21	7,860±613	58.1± .9	18.5± .4	57±6
2 percent of cod-liver oil	2	18	14	477.4±47.0	4.01± .10	70.8±3.9	45.38±3.31	8,080±631	56.2± .9	18.4± .4	57±6
2 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	2A	18	12	481.5±47.0	4.05± .10	71.1±3.8	44.93±3.31	7,925±651	55.7±1.0	18.0± .4	54±6
8 percent of cod-liver oil	3	18	12	568.4±48.4	3.48± .10	39.7±4.7	33.91±3.40	5,700±672	53.7±1.0	16.4± .4	41±6
8 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	3A	18	7	590.7±51.8	3.69± .10	36.7±5.7	29.56±3.79	5,075±722	54.8±1.0	16.8± .5	40±8

DATA THAT DO NOT SHOW STATISTICALLY SIGNIFICANT DIFFERENCES [2]

Source of vitamin D	Pen no.	Birds at— Beginning of experiment	Birds at— End of experiment	Average loss in live weight Feb. 13, 1934, to July 31, 1934	Average eggshell weight [8]	Average thickness of eggshells	Average strength of eggshells (drop of steel ball)	Average thickness of membrane of eggshells	Average total albumen weight per egg	Average percentage of thick albumen	Average fertility of total eggs set [9]
		Number	*Number*	*Grams*	*Grams*	*Millimeters*	*Centimeters*	*Millimeters*	*Grams*	*Percent*	*Percent*
1 percent of cod-liver oil	1	18	11	287.7± 84.0	6.73±0.15	0.319±0.007	15.86±0.38	0.040±0.002	33.6±0.8	60.30±1.35	79.1±6.0
1 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	1A	18	14	307.5± 74.5	6.57± .14	.307± .006	15.85± .33	.043± .002	34.1± .7	61.44±1.21	84.4±5.6
2 percent of cod-liver oil	2	18	14	322.9± 74.5	6.75± .14	.326± .006	16.90± .34	.041± .002	32.8± .7	58.83±1.21	78.7±5.2
2 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	2A	18	12	250.4± 80.4	6.63± .14	.315± .006	16.18± .36	.042± .002	32.2± .7	57.17±1.21	88.7±5.4
8 percent of cod-liver oil	3	18	12	301.3± 80.4	6.47± .14	.318± .006	16.57± .37	.042± .002	32.0± .7	60.79±1.25	88.0±6.8
8 percent of cod-liver oil plus 0.5 per cent of irradiated ergosterol 160 D	3A	18	7	438.6±105.3	6.25± .19	.324± .009	16.44± .39	.046± .003	32.6±1.0	58.52±1.66	87.5±8.1

[1] In this table standard errors, rather than probable errors, are used.
[2] According to Fisher's *z* test for significance.
[3] Date of maximum gain during the entire experiment.
[4] Average of the average weights of feed consumed.
[5] Weighted average of the percentage of hatchability of the fertile eggs set for the individual birds.
[6] Data for birds laying fewer than 5 eggs during the experiment were eliminated. Rest of data was adjusted by covariance analysis for the number of days each bird lived. The average number of days lived per bird for those birds from which data were used in this analysis was 308.5 out of a possible 336.
[7] Data adjusted by covariance analysis for the number of days each bird lived. The average number of days lived per bird for those birds from which data were used in this analysis was 313.8 out of a possible 336.
[8] Fresh, undried eggshells.
[9] Weighted average of the percentage of fertility of the total eggs set for the individual birds.

TABLE 2.—*Statistical significance of the differences found in table 1*

Sources of vitamin D compared [1]	Pen No.	Degree of significance in the results obtained in the experiment in respect to [2]—							
		Gain in live weight Aug. 29, 1933 to Feb. 13, 1934 [3]	Feed consumed per period of 4 weeks	Hatchability of fertile eggs	Eggs produced per bird	Weight of all eggs produced per bird	Egg weight	Yolk weight	Color of yolks
1 percent cod-liver oil and—	1								
1 percent cod-liver oil+irradiated ergosterol	1A	S	S	N	N	N	N	N	N
2 percent cod-liver oil	2	H	S	N	N	N	N	N	N
2 percent cod-liver oil+irradiated ergosterol	2A	H	N	N	N	N	N	S	N
8 percent cod-liver oil	3	N	H	H	N	S	H	H	H—[4]
8 percent cod-liver oil+irradiated ergosterol	3A	N	H	H	S	H	S	H	S
2 percent cod-liver oil and—	2								
8 percent cod-liver oil	3	N	H	H	S	S	N	H	S
8 percent cod-liver oil+ irradiated ergosterol	3A	N	S	H	H	H	N	S	S—[5]
1 percent cod-liver oil+irradiated ergosterol and—	1A								
8 percent cod-liver oil	3	N	H	H	S—[5]	S	H	H	S
8 percent cod-liver oil+irradiated ergosterol	3A	N	S	H	S	H	H	H	S—[5]
2 percent cod-liver oil+irradiated ergosterol and—	2A								
8 percent cod-liver oil	3	N	H	H	S	S	N	H—[4]	N
8 percent cod-liver oil+irradiated ergosterol	3A	N	H	H	H	H	N	S—[5]	N

[1] The differences are in favor of the first-mentioned source of vitamin D of each comparison.
[2] H=highly significant (odds of at least 99 to 1); S=significant (odds of at least 19 to 1); N=not significant.
[3] Date of maximum gain during the entire experiment.
[4] The odds are slightly less than 99 to 1.
[5] The odds are slightly less than 19 to 1.

The tables show that the birds in pen 1, which received 1 percent of cod-liver oil without irradiated ergosterol, laid eggs with significantly heavier yolks than those in pen 2A, which received 2 percent of cod-liver oil with irradiated ergosterol. The difference in the average weight of the yolks of the eggs from pen 2, which received 2 percent of cod-liver oil without irradiated ergosterol, and those from either pen 3 or 3A, was more significant than the difference in the average weight of the yolks of the eggs from pen 2A and those from either pen 3 or 3A.

The color of the yolks of the eggs from pens 1A, 2A, and 3A had a tendency to be lighter than the yolks of the eggs from pens 1, 2, and 3, but the differences were not statistically significant. Significant differences existed between the color of yolks obtained from either pen 1, 1A, or 2 and either pens 3 or 3A. The difference in yolk color between the eggs from pen 2A and either pen 3 or 3A was not statistically significant.

None of the differences in the loss of live weight of the birds, the weight, thickness, or strength of eggshells, the thickness of the shell membranes, the weight of the whole albumen, the percentage of the thick albumen, and the percentage of the fertility of the eggs, were statistically significant.

The data presented in this paper substantiate the conclusion drawn from previous work of Titus and Nestler (*33*) that excessive percentages of cod-liver oil in the diet of confined laying hens have a markedly deleterious effect on both egg production and hatchability. In the present work 8 percent of cod-liver oil in the diet of laying hens considerably lowered egg production, hatchability, average weight of eggs, total weight of eggs produced, weight of egg yolks, and weight of feed consumed. It also caused the yolks to have a lighter color.

A mixture of one-half percent of irradiated ergosterol 160 D, a quantity about four times that considered by Titus and Nestler (*33*) as necessary for laying birds, and 1, 2, or 8 percent of cod-liver oil, gave practically the same results as the same percentages of cod-liver oil when fed alone. This fact harmonizes with the observations of Barnes, Brady, and James (*1*) on the lack of effectiveness of a mixture of cod-liver oil and irradiated ergosterol in human nutrition, and those of Bethke, Record, and Kennard (*2*) on the lack of effectiveness of such a mixture for proper bone calcification in chicks.

SUMMARY

An experiment was carried on at the Agricultural Research Center at Beltsville, Md., in 1933–34, to compare the efficacy of cod-liver oil fed alone with that obtained by mixing it with irradiated ergosterol, as a source of vitamin D for confined laying hens. Six pens of crossbred pullets, the progeny of a mating of Rhode Island Red males with Barred Plymouth Rock females, were used. The birds were kept in strict confinement without access to direct sunlight. These six pens received the following vitamin D supplement: 1, 2, and 8 percent of cod-liver oil in the first three pens, and 1, 2, and 8 percent of cod-liver oil supplemented in each case by 0.5 percent of irradiated ergosterol 160 D in the last three pens. A phosphorus level of 1.2 percent and a calcium level of 3.0 percent were maintained throughout the experiment.

A mixture of 0.5 percent of irradiated ergosterol 160 D with 1, 2, and 8 percent, respectively, of cod-liver oil in the diet of laying hens showed no superiority over the same quantities of cod-liver oil without the addition of irradiated ergosterol.

The relatively high levels of vitamin D produced by combining cod-liver oil at the 1- and 2-percent levels with about four times the quantity of irradiated ergosterol necessary for laying chickens produced no deleterious effects on the hens or their eggs.

Cod-liver oil at the 8-percent level, whether supplemented with irradiated ergosterol or not, had a markedly deleterious effect on the production, average weight, total weight, and hatchability of the eggs; weight of the yolks; and the consumption of feed. It also caused the color of the yolks to be much lighter than those from birds receiving lower levels of cod-liver oil. However, it had no appreciable effect on the other items studied.

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