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The Chick's Requirement for 25-Hydroxycholecalciferol and Cholecalciferol[1]

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ABSTRACT Three experiments were conducted to compare the effect of 25-hydroxycholecalciferol (25-OHD_3) and cholecalciferol (D_3) on three week weight gains, feed utilization and tibia ash of broiler cockerels. In Experiment 1, vitamin D levels of 198 and 330 I.C.U./kg. from both vitamin D sources were fed at phosphorus levels of .40%, .45%, .50% and .55% furnished by sodium phosphate monobasic (SPM). Feeding 25-OHD_3 compared to D_3 significantly increased tibia ash if phosphorus levels were below .45%. No significant differences in tibia ash were found due to phosphorus levels when 25-OHD_3 was fed; however, when D_3 was fed a significantly larger tibia ash was found by feeding phosphorus above .40%. Chicks in Experiment 2 were fed .45% total phosphorus with either SPM or defluorinated phosphate as the phosphorus source and 25-OHD_3 or D_3 at dietary levels of 198 or 330 I.C.U./kg. Chicks fed SPM with either vitamin D source had improved weight gains and tibia ash if the diet contained a vitamin D level of 198 I.C.U./kg. Phosphorus source responses were equal if the diet contained 330 I.C.U./kg. of vitamin D. In Experiment 3, both vitamin D sources were fed at dietary levels of 66, 132, 198, 330 and 396 I.C.U./kg. in diets containing .45% total phosphorus with defluorinated phosphate as the phosphorus source. Tibia ash statistically equal to the maximum response was achieved with 132 I.C.U./kg. of 25-OHD_3 or 198 I.C.U./kg. of D_3. The potency of 25-OHD_3 was found to be 1.5 times greater than D_3 based on requirement levels of both vitamin D sources.

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INTRODUCTION

VARIOUS biologically active metabolites of cholecalciferol have been isolated (Blunt *et al.*, 1968a; DeLuca, 1973; Holick *et al.*, 1971; and Suda *et al.*, 1970a). The molecular structure of these metabolities are 25-hydroxycholecalciferol (25-OHD_3); 24, 25-dihydroxycholecalciferol; 25, 26-dihydroxycholecalciferol; and 1, 25-dihydroxycholecalciferol. Blunt *et al.* (1968b) found 25-OHD_3 to be more active than cholecalciferol (D_3) in promoting intestinal calcium

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transport and bone calcium mobilization and 25-OHD_3 was found to be 1.4 times more antirachitic for rats than D_3. Sunde (1975) concluded that 25-OHD_3 is effective in increasing bone ash and body weight at considerably lower levels for poultry than D_3. This researcher concluded that 320-400 I.C.U./kg. of feed 25-OHD_3 is required for maximum bone ash; whereas, 400 I.C.U./kg. D_3 is required. McNutt and Haussler (1973) reported that 1, 25-dihydroxycholecalciferol provides an effectiveness similar to 25-hydroxycholecalciferol, with both metabolites being between 1.5 and 2.2 times as active as cholecalciferol with respect to stimulation of weight gain and maintenance of plasma calcium levels. These researchers estimated the antirachitic potency of 1, 25-dihydroxycholecalciferol in chicks to be 1.3 times greater than cholecalciferol.

Edwards (1976) concluded that when a diet containing 0.6% calcium was fed, 200-400 I.C.U. of vitamin D_3/kg. of feed were adequate for maximum growth rate and calcification of bones as measured by bone ash; however, when a diet containing relatively low levels of calcium was fed 800 I.C.U. of vitamin D_3/kg. was not adequate to produce maximum growth rate or bone ash. Waldroup *et al.* (1963, 1965) reported that maximum growth rate and tibia ash could be obtained when chicks were fed diets containing only 0.50% calcium when 7920 I.C.U./kg. of vitamin D_3 was included in the diet. Similar weights and tibia ash were obtained with only 198 I.C.U. of vitamin D_3/kg. of diet when the diet contained 1.0% calcium. Edwards (1976) concluded that in view of our newer knowledge of vitamin D_3 and calcium metabolism it is difficult to rationalize the continued use of high calcium—low D_3 diets to meet calcium requirements when low calcium—high D_3 diets are adequate. Since economics greatly favor the latter in practical situations, it would seem logical to suspect that a more efficiently utilized source of vitamin D would also increase the utilization of calcium and phosphorus.

TABLE 1.—*Composition of basal*

Ingredient	Percent
Degerminated white corn, 30% germ	57.88
Soybean meal, 50% protein	35.40
Vegetable oil	3.00
Premix[1]	0.30
Salt	0.30
Methionine hydroxy analogue—Ca, 93%	0.12
Variable[2]	3.00
Total	100.00
Calculated analysis:	
Crude protein, %	22.79
Energy, M.E. Cal./kg.	3073
Total calcium, %	0.09
Total phosphorus, %	0.30
Methionine + cystine, %	0.83
Lysine, %	1.27

[1]Furnished the following amounts per kilogram of diet: Vitamin A palmitate, gelatin coated, 6614 I.U.; vitamin E, 2.2 I.U.; riboflavin, 4.4 mgs.; niacin, 27.6 mgs.; d-pantothenic acid, 8.8 mg.; folic acid, 275.6 mcg.; vitamin B_{12}, 8.8 mcg.; choline chloride, 551 mgs.; ethoxyquin, 55 mgs.; menadione sodium bisulfite complex, 2.8 mgs. or menadione sodium bisulfite, 1.7 mgs.; pyridoxine, 0.55 mgs.; manganese, 66.25 p.p.m.; zinc, 44 p.p.m.; iodine, 1.25 p.p.m.; iron (in sulfate form), 20 p.p.m.; copper (in sulfate form), 2 p.p.m.

[2]The dietary variables included the test calcium and phosphorus sources.

The chick's requirement for vitamin D_3 or cholecalciferol appears to be approximately 200 I.C.U./kg. (N.R.C., 1971). The purposes of these experiments were to compare the chick's requirement for 25-OHD_3 and D_3 and to study the effect of phosphorus source and level on utilization of 25-OHD_3 and D_3.

EXPERIMENTAL PROCEDURE

Broiler strain cockerels obtained from a commercial hatchery were used in three experiments to determine the chick's require-

TABLE 2.—*Effect of varying dietary levels of phosphorus and vitamin D from two vitamin D sources on chick growth, Exp. 1*

Vitamin D source[1]	Added vitamin D, I.C.U./kg.	Mean body weight, gms.[2] Total phosphorus, % .40	.45	.50	.55	Mean
D_3	198	394	413	394	408	402
D_3	330	406	410	410	398	406
	Mean	400	412	402	403	
25-OHD_3	198	379	394	395	398	392
25-OHD_3	330	398	408	424	386	404
	Mean	388	401	410	392	

[1] D_3, cholecalciferol; 25-OHD_3, 25-hydroxycholecalciferol.
[2] No significant differences.

TABLE 3.—*Effect of varying dietary levels of phosphorus and vitamin D from two vitamin D sources on tibia ash, Exp. 1*

Vitamin D source	Added vitamin D, I.C.U./kg.	Tibia ash, % Total phosphorus, % .40	.45	.50	.55	Mean[1]
D_3	198	30.76	33.91	33.94	33.87	33.12b
D_3	330	30.77	34.19	35.58	33.14	33.42b
	Mean[1]	30.76b	34.05a	34.76a	33.50a	
25-OHD_3	198	34.49	35.22	34.00	34.00	34.43a
25-OHD_3	330	34.26	34.78	35.63	35.19	34.96a
	Mean[1]	34.38a	35.00a	34.82a	34.60a	

[1] Means within a column or row and without a common letter are significantly different ($P < 0.05$).

ment for 25-hydroxycholecalciferol (25-OHD_3)[3] and cholecalciferol (D_3)[3]. At one day of age, the chicks were wingbanded and randomly assigned to decks in electrically heated battery brooders with raised wire floors. Test diets and tap water were furnished *ad libitum* in each experiment. Each test diet was fed to three lots of 10 broiler cockerels for a three week test period. Composition and calculated analyses of the basal diet are shown in Table 1.

In Experiment 1, added vitamin D levels of 198 and 330 I.C.U./kg. from both 25-OHD_3 and D_3 were fed at total dietary phosphorus levels of .40%, .45%, .50% and .55% furnished by sodium phosphate monobasic (SPM). Chicks in Experiment 2 were fed .45% total phosphorus with either SPM or defluorinated phosphate as the phosphorus source and 25-OHD_3 or D_3 at dietary levels of 198 or 330 I.C.U./kg. Experiment 3 was conducted to test the relative utilization of both vitamin D sources when fed at added vitamin D levels of either 66, 132, 198, 264, 330 or 396 I.C.U./kg. using SPM as the phosphorus source. Phosphorus was added at .45%. In all experiments the calcium:phosphorus ratios were held constant at 1.50:1 using calcium carbonate (U.S.P.) as the supplemental calcium source.

Group feed consumption and individual body weight data were obtained at three

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Table 4.—*Effect of different dietary sources of phosphorus and vitamin D on chick growth, Exp. 2*

Phosphorus source[1]	Added vitamin D, I.C.U./kg.	Mean body weight, gms.[2] Vitamin D source D_3	$25\text{-}OHD_3$	Mean
SPM	198	413	394	404a
SPM	330	410	408	409a
	Mean	412a	401a	
DP	198	376	374	375b
DP	330	383	389	386ab
	Mean	379b	381a	

[1] SPM, sodium phosphate monobasic; DP, dicalcium phosphate.
[2] Means within a column and without a common letter are significantly different (P < 0.05).

Table 5.—*Effect of different dietary sources of phosphorus and vitamin D on bone ash, Exp. 2*

Phosphorus source[1]	Added vitamin D, I.C.U./kg.	Tibia ash, %[2] Vitamin D source D_3	$25\text{-}OHD_3$	Mean
SPM	198	33.91	35.22	34.56a
SPM	330	34.19	34.78	34.49ab
	Mean	34.05a	35.00a	
DP	198	32.11	32.42	32.26c
DP	330	32.26	33.43	32.84bc
	Mean	32.18b	32.92b	

[1] SPM, sodium phosphate monobasic; DP, dicalcium phosphate.
[2] Means within a column and without a common letter are significantly different (P < 0.05).

Table 6.—*Effect of vitamin D source and level on chick body weight and tibia ash, Exp. 3*

Added vitamin D, I.C.U./kg.	Chick weight, gms. Vitamin D source D_3	$25\text{-}OHD_3$	Mean[1]
66	352	362	357b
132	377	389	383ab
198	376	374	375ab
264	378	386	382ab
330	383	389	396ab
396	397	383	397a
Mean[2]	378	383	—
Added vitamin D, I.C.U./kg.	**Tibia ash, %[1] Vitamin D source D_3**	**$25\text{-}OHD_3$**	**Mean**
66	31.04b	30.32b	30.68d
132	31.04b	32.01ab	31.53cd
198	32.11ab	32.42ab	32.26bc
264	32.05ab	33.27a	32.66abc
330	32.26ab	33.43a	32.84ab
396	33.89a	33.96a	33.88a
Mean[2]	32.07	32.55	—

[1] Means within a column and without a common letter are significantly different (P < 0.05).
[2] No significant differences.

weeks of age. Since no consistent differences in feed efficiency were found, these data are not shown. At the end of each experiment, all chicks were sacrificed. A minimum of 10 tibia per lot was taken for bone ash determinations. Each group of fresh tibia was boiled for 3-5 minutes in distilled water, cleaned with the aid of a knife and cheesecloth, ether-extracted, and dried for 24 hours. The fat-free, moisture-free tibia bones were randomly pooled into two lots of five tibia each and ashed at 600° C. for eight hours. For each experimental diet, there was a total of six ashings.

A factorially arranged randomized complete block experimental design was used in each experiment. Statistical examinations of the data were performed using the analysis of variance (Steel and Torrie, 1960). Duncan's new multiple range test (1955) was used to separate significant differences between means. All statements of significance refer to the 5% level of probability.

RESULTS AND DISCUSSION

Experiment 1. No significant differences in chick weights were found due to vitamin D source and level when dietary phosphorus levels were varied, as shown in Table 2. No significant differences were found due to varying phosphorus levels. Waldroup *et al.* (1963) concluded that bone ash is more responsive to alterations of dietary calcium, phosphorus and vitamin D_3 than is body weight. Feeding 25-hydroxycholecalciferol (25-OHD_3) resulted in a significantly increased tibia ash when compared to cholecalciferol (D_3) if the phosphorus level was below .45% (Table 3). No significant differences in tibia ash were found due to vitamin D source at phosphorus levels of either .45%, .50%, or .55%. Apparently 25-OHD_3 is more effective than D_3 in aiding the absorption of calcium and indirectly phosphorus and, therefore, increasing bone ash when calcium and phosphorus levels are low. This effect was evident at more critical levels of phosphorus (.40%) but was not apparent when higher dietary levels of phosphorus were fed. No significant differences in bone ash were found due to vitamin D levels.

Experiment 2. Chicks fed D_3 as the vitamin D source had significantly increased weight gains (Table 4) and tibia ash (Table 5) when the diet contained sodium phosphate monobasic (SPM) as compared to defluorinated phosphate (DP). However, no significant weight differences due to source of phosphorus were found when 25-OHD_3 was fed as the vitamin D source (Table 4). Using SPM as the phosphorus source gave a significantly larger tibia ash using either vitamin D source (Table 5) when compared to DP. This finding confirms the increased effectiveness of 25-OHD_3 as compared to D_3 as was apparent in Experiment 1. In general, no significant differences in either vitamin D source or level were found, although the vitamin D source effect approached significance. A numerically higher tibia ash was found by feeding 25-OHD_3 as compared to D_3.

Waldroup *et al.* (1963, 1965) reported that maximum growth rate and tibia ash could be obtained when chicks were fed diets containing only 0.50% calcium when 7920 I.C.U./kg. of D_3 was included in the diet. Similar weights and tibia ash were obtained with only 198 I.C.U. of D_3/kg. when the diet contained 1.0% calcium. In view of this research, it would seem apparent that the more potent 25-OHD_3 is needed to maximize bone ash at lower levels of calcium and phosphorus as shown in Experiments 1 and 2.

Experiment 3. Significantly larger body weights were observed by feeding 396 I.C.U.lhkg. vitamin D as compared to 66 I.C.U./kg. (Table 6). Tibia ash was slightly increased at each dietary vitamin D level; however, no significant differences were found due to vitamin D source. Tibia ash significantly increased due to increased vitamin D levels using either vitamin D source. The chick's requirement for vitamin D based on tibia ash varied with the vitamin D source. Chick's vitamin D requirement using cholecalciferol (D_3) as the vitamin D source was 198 I.C.U./kg., which confirms the N.R.C. (1971) requirement of 200 I.C.U./kg. However, 132 I.C.U./kg. apparently met the chick's vitamin D requirement when 25-OHD_3 was used as the vitamin D source. Sunde (1975) also concluded that 25-OHD_3 was more effective at lower levels for poultry than D_3. If the requirement levels are considered in determining potency of vitamin D sources, the potency of 25-OHD_3 is 1.5 times greater than D_3. These results compare favorably with those of Blunt *et al.* (1968b) who concluded that 25-OHD_3 was 1.4 times more active than D_3 in antirachitic activity when administered orally.

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NEWS AND NOTES

(Continued from page 505)

Sanford's community service has mirrored his University service, and that service has been described as "dependable," "unselfish," "meticulous," and "dedicated."

A sampling of his contributions:

In political affairs he has served on the Riley County Republican Party Central Committee and been Precinct Committeeman.

In religious affairs he has been a Deacon and Elder of the First Presbyterian Church.

In educational affairs he has been President of the High School P.T.A.

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